EFFECTS OF CLOSE-UP DIETARY ENERGY STRATEGY AND PREPARTAL DIETARY MONENSIN ON HEPATIC mRNA EXPRESSION OF ENZYMES INVOLVED IN GLUCOSE AND LIPID METABOLISM IN TRANSITION COWS

BY

SHUOWEN CHEN

THESIS

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Master's Committee:

Professor James K. Drackley, Chair
Associate Professor Juan J. Loor, Director of Research
Professor Walter L. Hurley

ABSTRACT

A two-stage feeding system for dry cows (far-off plus close-up groups) has been used by farmers for a long time. However, little basic research has been conducted to evaluate the effects of such approach on liver function. The objective of this study was to 1) to compare the effect of two different diets: one-stage controlled-energy high fiber diet (CON) fed throughout the entire dry period versus a two-stage diet (OVER) with controlled-energy in far-off period followed by higher energy in close-up period; and 2) to evaluate the effect of monensin (M) supplementation to these two different diets on bovine hepatic mRNA expression of enzymes involved in liver adaptive mechanisms during the transition period.

The treatments were arranged in a 2 (one-stage CON or two-stage OVER feeding strategy) × 2 (inclusion of 0 or 22g/ton of M) factorial arrangement. The CON diet was formulated for a dietary energy concentration of 1.30 Mcal NE_L/kg DM and the OVER diet for 1.49 Mcal NE_L/kg DM. We calculated dry matter intake and analyzed serum NEFA and BHBA concentration and liver composition of the cows both prepartum and postpartum. We also examined mRNA expression at -14 and 7 via qPCR of gluconeogenesis genes (*FBP1, PC, PCK1*), fatty acid oxidation and ATP synthesis genes (*CPT1A, ACOX1, ATP5G1*), lipid metabolism genes (*APOA1, APOB, FADS2, SCD, HMGCR*), inflammation, acute phase response, and detoxification/oxidative stress genes (*TNF, IL6, HP, GSTM1*), growth hormone/IGF-1

axis genes (*GHR, IGF1*) and transcription factors (*FOXA2, HNF4A, NR4A1, SREBPF2*).

In cows fed the CON diet, serum NEFA concentrations were higher at -2 and -1 week relative to calving and serum BHBA concentrations were also higher at -2 wk. We also observed a lower concentration of BHBA in cows fed monensin at 2 wk. Liver compositions and DMI were not affected by any of the treatments. Cows fed the CON diet had an up-regulated expression of *CPT1A, APOA1, PC, PCK1* and a down-regulated expression of *ATP5G1, SCD* and *GHR* at -14 d. At 7 d, expression of *GHR* and *IL6* was lower in cows fed CON diet than those fed OVER diet. Inclusion of monensin resulted in an increase in expression of *SCD* at 7 d and a decrease in *APOA1* at -14 d in cows compared with the control group. Expression of *IGF1* and *GSTM1* was increased by monensin at -14 d.

Based on these results, we conclude that the two-stage diet (OVER) had no advantage compared with the one-stage controlled energy-high fiber diet (CON) and inclusion of monensin had very little benefit in improving hepatic adaptations to metabolic changes in transition cows.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

BCS	body condition score
BHBA	β-hydroxybutyrate
BW	body weight
CON	controlled energy high forage diet
CPT	carnitine palmitoyl transferase
CRC	controlled release capsule
DIM	days in milk
DM	dry matter
DMI	dry matter intake
EB	energy balance
DP	dry period
FA	fatty acid
g	gram(s)
kg	kilogram(s)
LCFA	long chain fatty acids
ml	milliliter
mM	millimolar
mmol	millimole
M	monensin

N	nitrogen
NEB	negative energy balance
NEFA	nonesterified fatty acids
NRC	National Research Council
NE_L	net energy of lactation
OVER	controlled energy followed by higher energy diet
TAG	triacylglycerol
TF	transcription factor
TG	triglycerides/triacylglycerol
TMR	total mixed ration
VFA	volatile fatty acid
VLDL	very low density lipoproteins
wk	week(s)

CHAPTER 1

LITERATURE REVIEW

The importance of a successful transition from late pregnancy to early lactation is well accepted by most researchers and dairy industry managers. The transition period or periparturient period has been defined as 3 week before calving to 3 week after calving and is the time when most health disorders occur (Drackley 1999). Disorders such as milk fever, ketosis, retained placentas and displaced abomasum normally occur during the transition period and are associated with the severity of negative energy balance. In addition, it is during this period in which the cow has a greater demand for energy and, in most cases, accompanied with a decrease in voluntary dry matter intake (Petersson-Wolfe, Leslie et al. 2007).

During the last 3 weeks of pregnancy, nutrient demands by the fetal calf and placenta reach their maximum (Bell 1995). After calving, the initiation of milk synthesis and rapidly increasing milk production greatly increases mammary demands for glucose for milk lactose synthesis. Dry matter intake decreases gradually, therefore nutrient supply is not able to match the abrupt increase in nutrient requirements at parturition and the initiation of lactation. The deficit between total dietary energy intake and energy requirements has to be made up by fat mobilization from adipose tissue (Drackley 1999). When stored fat is mobilized, blood NEFA concentrations are elevated up to twofold between 17 and 2 d prepartum (Grummer, Hoffman et al. 1995). Blood NEFA concentration is often referred to as a valuable

reflection of the degree of adipose tissue TG mobilization and a tool to evaluate energy balance; therefore, as negative energy balance becomes more severe, more NEFA are released from body fat and the concentration of NEFA in blood increases. Elevated blood NEFA concentration prepartum is often linked to increased risk of metabolic disorders and health problems in postpartum period in dairy cows.

NEFA and BHBA

As the concentration of NEFA in blood increases around calving and in early lactation, more NEFA are taken up by the liver (Reynolds, Aikman et al. 2003). In the liver, NEFA can be completely oxidized to carbon dioxide to provide energy for the liver which uses LCFA oxidation as its major source of energy. When the amount of NEFA which is taken up by the liver exceed its capacity to process fatty acids, they are converted to ketone bodies such as BHBA and acetoacetate which are released into the blood and serve as fuels for other tissues, such as muscle (Goff 2006). NEFA mobilized from adipose tissue due to negative energy balance can also be reconverted to TG, though at a low rate inherently. However, the hepatic tissue capacity to esterify NEFA to TG is increased at the time of parturition (Litherland, Dann et al. 2011). Increased TG concentration in the liver may lead to lipid accumulation and ultimately fatty liver may develop when the limit for fatty acids to be oxidized and secreted as TG in very low density lipoproteins is reached. This TG accumulation usually reaches a maximum between 7 and 14d after parturition (Van den Top, Geelen et al. 1996).

Dairy cows rely on hepatic gluconeogenesis to meet their glucose requirements for milk synthesis at a time when DMI usually does not reach its maximum.

Propionate production during the early postpartal period is insufficient to synthesize the total amount of glucose needed due to a decline in DMI (Drackley 2001). Amino acids from the diet or from skeletal muscle breakdown as well as glycerol from mobilized body fat must serve as resources to account for most of the remaining glucose synthesis (Reynolds, Aikman et al. 2003). The negative energy balance and glucose insufficiency postpartum also lead to increased production of ketone bodies in the liver, which serve as a way to spare the glucose for milk synthesis and can result in clinical or subclinical ketosis (Duffield 2000).

DMI

It is of critical importance to meet nutrient requirement in the transition period by an optimal nutrient supply which determines the production and performance of cows. Therefore, DMI is the most important variable in that sense (Drackley, Donkin et al. 2006). But there is a delay in the nutrient supply to meet requirements for milk synthesis: milk production peaks 5 to 7 weeks but DMI peaks later around 8 to 22 weeks postpartum (Ingvartsen and Andersen 2000). Demands for glucose by Holstein cows increases from 1000 to 1100 g/d 21 d prepartum to over 2500 g/d by 21 d postpartum (Overton, Emmert et al. 1998). However, DMI may decrease by 30% from 5 to 7 d prepartum. Researchers calculated that between thirty four weeks of gestation and one week postpartum there is a three time increase in the demand for glucose, two time for amino acids (AA), and approximately five times for fatty acids. It was concluded that demand for glucose, amino acids, and fatty acids in high-producing dairy cows during early lactation cannot be merely met by DMI (Bell

1995).

Regulation of voluntary DMI during the transition period is complex and largely not understood (Litherland, Dann et al. 2011). As parturition approaches, the high circulating estrogen may be one factor that contributes to decreased DMI (Grummer 1993). Relationships between prepartum and postpartum DMI and the accumulation of lipid in the liver after calving were analyzed (Drackley 2003). It was concluded that changes in DMI prepartum are more highly related to poor intakes and fatty livers after calving than DMI per se. Similar conclusions have been reached by others: Changes in prepartum DMI may affect postpartum DMI more than absolute DMI prepartum (Douglas, Overton et al. 2006). In this way, DMI may be referred to as an indicator of the overall comfort and well-being of the transition dairy cows.

Decreased DMI during the week before calving leads to TG accumulation in the liver (Drackley, Donkin et al. 2006). The magnitude of negative energy balance is much greater early postpartum than prepartum, which causes mobilization of fats to provide the energy required for milk production (Goff 2006). Therefore, one of the main focuses of diet management before calving is to minimize the duration and magnitude of DMI decrease.

Controlled-energy and high fiber diet

Preventing excessive energy intake during the dry period can have positive effects on NEB postpartum and liver TG accumulation. Various researchers have found a potential negative effect of excessive energy intake prepartum. It can lead to severe metabolic burden in liver of transition cows postpartum (Vandehaar, Yousif et

al. 1999) or lead to lower postpartum DMI and metabolic imbalance even when body condition score (BCS) does not indicate over-conditioning (Drackley 2005). On the other hand, lower energy intake during the far-off dry period led to higher DMI, greater energy balance, and lower serum NEFA and BHBA during the first 10 DIM, and total lipids and TG in the liver were lower at 1 DIM while cows fed ad libitum during the close-up period tended to have lower energy balance and lost more body condition (Dann, Litherland et al. 2006). Other research also supports this observation with lower postpartum concentrations of total lipid and TG in the liver of cow feed-restricted prepartum (Douglas, Overton et al. 2006). Another study also found a greater rate of hepatic fatty acid esterification but lower rate of β-oxidation in cows overfed with energy prepartum compared with cows that were energy-restricted to ~80% of requirement (Litherland, Dann et al. 2011).

A controlled energy, high fiber diet should be balanced to provide adequate metabolizable protein, minerals, and vitamins, without supplying excessive energy (Drackley 2005). Other researchers also demonstrated the positive effects of controlled-energy diet on not only DMI but also milk yield postpartum. When cows were restricted in DMI prepartum, DMI and milk yield were slightly higher postpartum and high DMI prepartum had no advantage (Holcomb, Van Horn et al. 2001). Others concluded that cows were more successful in terms of serum NEFA concentration and liver fat content postpartum with increased DMI even with a lower BCS prepartum caused by restricted energy intake (Overton 2004).

The two-stage feeding strategy

Most of the research has focused on dividing the DP into two stages: stage 1, "far-off," which starts when the cow has been dried off and continues to about 21 d before calving, and stage 2, "close-up," which is usually the last 3 wk of gestation. The NRC (2001) recommended that a diet providing 1.25 Mcal/kg of NE_L be fed for the first 40 d of the dry period, and a diet providing 1.54 to 1.62 Mcal/kg of NE_L be fed for the last 21 d before calving. One reason given for using a two-stage diet is to prepare cows for high concentrate diets following lactation. Overton and Waldron (2004) concluded that two-stage feeding strategies for dry cows would prevent overfeeding during the first part of the dry period and facilitate metabolic adaptation during the late dry period. Some other studies have found that feeding higher energy prepartum was beneficial. Cows fed a higher energy density diet consumed more NE_L and gained more body condition (Vandehaar, Yousif et al. 1999). Cows fed low forage diets did not metabolize as much energy from fat stores as cows fed higher forage (Holcomb, Van Horn et al. 2001).

However, very few studies working on transition cows discussed how cows were fed during the far-off period, which limits the interpretation of results (Dann, Litherland et al. 2006). In some cases, cows would still have higher energy intake relative to their requirements due to the diet density formulated to limit energy intake and not DMI (Janovick and Drackley 2010). Information on what cows were fed in the far-off dry period has often been lacking in studies on close-up diets.

Research has revealed that feeding strategy in the far-off dry period may be more

important than that in the close-up period. A study was conducted aiming to evaluate the effect of far-off diets relative to close-up diets in regard to transition metabolism and performance of dairy cows (Dann, Litherland et al. 2006). The three far-off diets were formulated to meet 80, 100, or 150% of the NE_L requirements set by NRC for this period. The two close-up diets (fed to all cows 24 d before estimated calving) were either fed ad libitum to ensure at least 140% of the NE_L or was restricted-fed to provide 80% of the NE_L required according to the NRC (2001). After calving, all cows were fed the same lactation diet.

Results were quite intriguing in that cows fed 150% of their required NE_L had lower DMI and EB, but higher NEFA and BHBA concentrations during the first 10 d of lactation. Overall, cows fed 150% of the required NE_L had the greatest NEFA concentration whereas those fed 80% of the required NE_L had the lowest. Regarding the close-up diets and their interaction with the far-off diets, no significant difference was found. Therefore, they concluded that the feeding strategy during the far-off period was more important and conferred more negative effects than the differences in the close-up diets on transition period metabolism of dairy cows.

In another study (Janovick, Boisclair et al. 2011), researchers found similar results. They fed cows two diets: a controlled-energy high-fiber diet versus a higher-energy diet fed throughout the dry period. Cows fed the controlled-energy diet had a smaller decrease in DMI than cows fed the higher-energy diet before calving, had less lipid accumulation in the liver, and had lower concentrations of BHBA in the blood.

In a study using genomic approaches to measure bovine hepatic gene expression in response to restricted and non-restricted energy intakes, they also measure the concentrations of NEFA and BHB and found out that their concentrations increased gradually for both treatments, but both concentrations from the non-restricted treatment were numerically lower during the dry period and significantly higher after calving (Loor, Dann et al. 2006).

However, the effects of prepartum feeding strategy could be more complicated due to other factors in cows. For example, researchers found that for primiparous cows, there were no difference in variables measured prepartum and postpartum among three DP diets which met 80%, 100% and 150% of the NE_L requirement respectively whereas multiparous cows fed the high energy diet had lower DMI during in the first 3 wk postpartum (Janovick and Drackley 2010).

Therefore, research is still needed to compare a one-stage low energy, high fiber diet fed throughout the entire dry period to a two-stage diet currently used by many dairy farmers.

Monensin

Monensin (M), an ionophore, is a carboxylic polyether that is produced naturally from a *Streptomyces cinnamonensis* strain. Ionophores are feed additives that have been used since 1977 in the beef industry to improve feed efficiency and prevent coccidiosis (Petersson-Wolfe, Leslie et al. 2007). By forming a lipophilic complex with cations, ionophores facilitate their transport across membranes (Martineau, Benchaar et al. 2007). Altering rumen fermentation pattern is the primary action of

monensin and it favors increasing propionate production rate and concentration in the rumen (Green, McBride et al. 1999). Greater propionate supply leads to increased hepatic gluconeogenesis (Aiello and Armentano 1987) and sometimes with a corresponding decrease in NEFA and BHBA in plasma (Overton 2004). Therefore, the primary benefit of monensin supplementation is often assumed to be the mitigation of the negative energy balance and increasing in the supply of glucose to the transition cow.

Monensin alters the rumen micro-flora by selectively inhibiting the growth of bacteria which produce acetate and hydrogen via disturbing their protein synthesis, resulting in an increase of the rumen bacteria that will increase the production of propionate, which is the primary glucose precursor (Dubuc, DuTremblay et al. 2009), therefore further decreases adipose tissue mobilization and fat infiltration of the liver (Chung, Pickett et al. 2008). In addition, monensin also reduces methane production in the rumen, and reduces circulating concentrations of total ketone bodies, as well as decreases displaced abomasum, and related disorders (Rogers and Hope-Cawdery 1980; Sauer, Kramer et al. 1989).

Many studies have been conducted regarding the various effects of monensin supplementation on metabolic parameters of dairy cows in different stages of lactation. Monensin-treated cows had lower plasma concentrations of glucose, free fatty acid (FFA), and beta-hydroxybutyrate (BHBA) than did control cows before calving. However, no significant differences in plasma concentrations of glucose, FFA, and BHBA were found between groups after calving (Stephenson, Lean et al. 1997). On

the contrary, another study found that monensin treatment significantly reduced serum BHBA concentrations at week 1, 2, and 3 postpartum and significantly raised serum glucose concentrations during week 1 and 2 of lactation (Duffield, Sandals et al. 1998). Similarly, another study using controlled-release capsule (CRC) monensin treatment demonstrated that monensin decreased the ruminal acetate to propionate ratio, decreased the BHBA concentration, and increased pH (Green, McBride et al. 1999). Besides, monensin supplementation was effective in increasing concentration of triglyceride-rich lipoproteins in blood during the postpartum transition period of dairy cows. This suggested a role for M in reducing the risk of hepatic lipidosis and other diseases resulting from metabolic disturbances of cows during the transition period (Mohebbi-Fani, Nazifi et al. 2006).

For the effect of monensin on DMI and BW, some researchers observed no change DMI or BW in cows fed monensin during lactation (Martineau, Benchaar et al. 2007). The effect of monensin treatment may be due to other factors especially administration methods. As reviewed before, monensin treatment in most studies were given by CRC administration method and it appeared to have a significant effect on various metabolic parameters of cows, while very little is known about the effect of mixing monensin into the total mixed ration (TMR) throughout the entire dry period. Besides, very few studies have used genomic approaches such as qPCR to evaluate the effect of monensin on transition metabolism of dairy cows in terms of gene expression, while most studies on monensin treatment effect were conducted *in vivo*.

Research is needed to evaluate the effect of monensin supplemented with

controlled-energy or higher-energy diet throughout the dry period on metabolites in transition cows and genomic approaches are required in the research to measure gene expression under different treatments in order to better understand its role in transition metabolism.

Adaptations in liver in transition cows

Tremendous metabolic and endocrine adjustments must be made to support a successful transition of the dairy cows from late gestation to lactation. The liver sits at the crossroads of metabolism and plays a critical role in coordination of nutrient fluxes in support for pregnancy and lactation as requirements for glucose and metabolizable energy increases two or three times from -21 to 21 after parturition. The liver must adapt quickly to provide the increased glucose for milk production and to uptake increased NEFA extensively mobilized from adipose tissue (Drackley 2001).

The primary energy source for liver function is not glucose oxidation but LCFA, lactate, butyrate, valerate, the branched-chain VFA, and acetyl-coenzyme A (CoA) produced from catabolism (Drackley 2001). Drackley et al. (1991) demonstrated that *in vitro* the proportion of total utilization of NEFA by liver slices represented by oxidation increased as energy balance decreased, whereas the proportion of total flux that was esterified to TG decreased. (Drackley, Beitz et al. 1991). As the concentration of NEFA in blood increases around calving or in early lactation, more NEFA are taken up by the liver and hepatic oxidation rates will increase (Drackley 2001).

If the rate of influx of NEFA exceeds that of fatty acid oxidation and TG secretion

in the liver, increased TG synthesis and accumulation would occur. Therefore, the hepatic capacity of fatty acid oxidation becomes one of the important factors that regulate the disposition of NEFA. Ad libitum feeding of high-energy diets during the dry period can increase esterification capacity and decrease oxidation capacity in liver at 1 d postpartum (Litherland, Dann et al. 2011). Other researches also presented the evidence for negative effects of dry period overfeeding on hepatic enzymes of NEFA oxidation which support our previous discussion on feeding strategies (Murondoti, Jorritsma et al. 2004).

A key site for fatty acid oxidation is in the mitochondria, therefore the primary control point seems to be at the mitochondrial membrane which determines whether NEFA enter the mitochondria for oxidation or are converted to TG via microsomal esterification (Zammit 1999). The enzyme carnitine palmitoyltransferase (CPT-1) controls the entry of NEFA into the mitochondria for oxidation to carbon dioxide or ketone bodies. Activity of CPT-1 in ruminants is inhibited by malonyl-CoA, which is produced during metabolism of propionate (Knapp 1990).

The activity of CPT-1 and its key inhibitor malonyl-CoA during the periparturient has received substantial attention. Activity of CPT-1 was shown to be greater at 1d and 21d postpartum than -21d prepartum (Dann and Drackley 2005). Overfeeding during the dry period also made CPT-1 more sensitive to inhibition by malonyl-CoA (Dann and Drackley 2005). Malonyl-CoA concentration is responsible to changes in insulin and glucagon in ruminants (Brindle, Zammit et al. 1985), increasing when insulin increases. For microsomal esterification, our lab also determined that the

hepatic mRNA abundance for mGPAT, as an esterification enzyme, decreased during the dry period to 1 d after calving and then increased sharply at 14 d postpartum (Loor, Dann et al. 2005).

An alternative pathway of fatty acid oxidation occurs in peroxisomes. Inducing peroxisomal β-oxidation may provide an oxidative pathway to deal with excess uptake of NEFA around parturition (Douglas, Overton et al. 2006). It serves as a contributing factor to lower hepatic accumulation of TG when it may oxidize the large influx of mobilized NEFA and such pathway may be a component of the adaptations of fatty acid metabolism in liver for ruminants during the periparturient period (Drackley 2005).

As discussed above, the increased requirement for glucose by mammary gland at calving to initiate lactation cannot be made up by feed intake and propionate supply, therefore triggering gluconeogenesis from other sources other than propionate produced in rumen fermentation. Under this condition, Alanine, glutamine and glycerol from body fat mobilization are considered to be major sources for gluconeogenesis. After calving, it seems like those glucogenic amino acids contribute more than propionate to produce glucose. Researchers have observed three-fold increase in rates of muscle protein mobilization during the first week after calving compared with prepartum values (Overton 1998). Moreover, the capacity of liver tissue to convert alanine to glucose was significantly higher than that to convert propionate to glucose after calving (Overton 1998), but it seems that prepartum intake had no effect on the conversion of alanine to glucose.

It was suggested that pyruvate carboxylase (PC), as an important enzyme in gluconeogenesis which creates oxaloacetate from pyruvate, was increased 7.5 fold at 1 postpartum compared values at -28 d and then decreased to prepartum values by d 28 postpartum (Greenfield, Cecava et al. 2000). In contrast, another key regulatory enzyme involved in gluconeogenesis such as PEPCK changed in a similar manner in the liver, though more slowly. Mitochondrial PEPCK is not responsive to hormones or physiological state (Greenfield, Cecava et al. 2000) which supports the results of another study (Overton 1998).

CHAPTER 2

INTRODUCTION

The weeks surrounding parturition are a critical time in the life cycle of a high-producing dairy cow. During this period, cows make many metabolic and physiological adjustments to support the transition from pregnancy to lactation. Profitability of a cow is dependent on transition period success; however, nearly 50% of cows have at least one adverse health event during the transition period (Drackley 2005). Periparturient diseases of transition cows caused by metabolic disorders are due to failure to adapt to metabolic and physiological changes and cows become susceptible to certain infectious pathologies in that period.

Prepartum dietary management for dairy cows has been an important focus amongst investigators. The main objective of prepartum feeding is to minimize the decrease of dry matter intake (DMI) and maximize energy intake around parturition. Many researchers have investigated the effect of different diets in dry-off or close-up period on periparturient metabolism and performance of dairy cows. It has also been demonstrated that what is fed through the far-off dry period may be more important than what is fed during the final 3 wk prepartum (Dann 2006).

Liver is critical to many of the processes occurring around parturition in dairy cows, including gluconeogenesis and metabolism of fatty acids mobilized from adipose tissue. Negative energy balance caused by the deficit between high energy requirement and decreased total energy intake is accompanied by elevated serum

non-esterified fatty acids (NEFA) and elevated liver uptake of NEFA. Fat accumulation may impair normal functions of the liver. Liver functions are considered to be controlled through the coordinated expression of a large number of genes. The difference in hepatic gene expression pattern of dairy cows fed moderate dietary energy ad libitum or restricted during the entire dry period may help to better understand the complex metabolic adaptations in transition cows (Loor, Dann et al. 2006). The genomics approach may help identify regulatory mechanisms in liver during the transition period (Loor, Dann et al. 2005).

Monensin administration can improve energy balance by improving the supply of glucose precursors such as propionate available to the cow (Arieli, Dicken et al. 2008). Monensin has been reported to decrease plasma concentrations of glucose, NEFA, and BHBA before calving (Stephenson, Lean et al. 1997). Research is needed to evaluate the results of mixing monensin into the total mixed ration (TMR) throughout the entire dry period.

The objectives of this study were 1) to compare the effect of two different diets: one-stage controlled-energy diet versus a two-stage diet (far-off controlled-energy followed by close-up higher energy) and 2) to evaluate the effect of monensin added to these two different diets on hepatic mRNA expression of enzymes related to glucose and lipid metabolism in transition cows.

CHAPTER 3

MATERIALS AND METHODS

Animal and diets

The study was conducted at the University of Illinois Dairy Research and Teaching Unit. All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee (protocols number 08179 and 08225).

The trial was a randomized design with a 2 × 2 factorial arrangement of treatments. The four dry period treatments consisted of a single controlled-energy feeding program or a two- stage dry period feeding program, each without or with inclusion of monensin. After calving, all cows were offered the same lactation ration, containing monensin.

Thirty-one Holstein dairy cows were used in the experiment. Cows were randomly assigned to treatments. Lactating cows were dried off at least 50 days prior to expected parturition and moved to the experimental freestall barn. Cows entering their first lactation were moved into the experimental freestall barn at least 50 days prior to expected parturition. After parturition, all cows will be moved into individual tiestalls.

At the day cows were dried off, they were moved to the freestall pens and assigned a Calan feed gate (American Calan, Northwood, NH). All cows began receiving the experimental diets immediately and data were collected, but only the last 21 d of the far-off period were used for statistical analysis due to the variability in

length of this period. For the close-up period, only the last 14 d before calving were analyzed for the same reason. Three days before the estimated calving date or as calving signs appeared, cows were moved to individual concrete-floored calving pens (bedded with straw) located within the freestall pens. Immediately after calving, cows were moved to a tie-stall barn and were assigned an individual stall for the duration of the study.

Diets were balanced according to NRC (2001) recommendations. The controlled-energy diet was formulated for a DMI of 12.2 kg/d with an energy density of 1.30 Mcal NE_L/kg DM. For the two-stage program, the same diet was fed for the far-off stage (from the day the cow was dried to 21 d before the estimated day of calving) and a close-up diet was fed during the close-up stage (from 21 d before estimated day of calving to actual day of calving). This diet was designed to be intermediate in ingredients and nutrient profile to far-off and lactation diets. The close-up diet was balanced for DMI of 10.5 kg/d and an energy density of 1.49 Mcal NE_L/kg DM. Each of the two dry period diets were formulated both without and with monensin at a target of 22 g/ton (24.2 g/metric ton) of total dietary DM. After calving, all cows were fed the same lactation diet that was formulated for a DMI of 22.7 kg/d and an energy density of 1.70 Mcal NE_L/kg DM. The lactation diet contained monensin at 14 g/ton (15.4 g/metric ton) of dietary DM.

All diets were mixed in a Keenan 350 TMR mixer wagon using PACE software and Mech-Fiber technology (Richard Keenan & Co., Borris, Co. Carlow, Ireland). All ingredients were sampled weekly for determination of DM content to adjust ration

formulation. After weekly adjustment of the rations to maintain the desired DM ratio of ingredients, the ration DM content was adjusted to 46% for all dry period diets by addition of water daily when diets were mixed. No water was added to the lactation diet.

Blood samples were obtained by puncture of a tail vein or artery three times weekly (Monday, Wednesday, and Friday) from all cows before the morning feeding. Serum aliquots were obtained within 4 h from the time the last sample was collected. Serum was stored in a freezer at -20 ℃. At the end of the trial, samples were identified based on time relative to actual calving that corresponded to one sample per week during the far-off period (up to 4 wk, sample from mid-point of week used) and samples that corresponded to -13 d (-14 to -12 d), -10 d (-11 to -9 d), -7 d (-8 to -6 d), -4 d (-5 to -3 d), and -1 d (-2 or -1 d) relative to calving during the close-up period. Postpartum, samples were identified that corresponded to +1 d (+1 or +2 d), +4 d (+3 to +5 d), +7 d (+6 to +8 d), +10 d (9 to +11 d), +13 d (+12 to +14 d), +16 d (+15 to +17 d), and +19 (+18 to +20 d) relative to calving, and then one sample per week from wk 4 through wk 9. These samples were analyzed for concentrations of BHBA and NEFA by autoanalyzer methods at the University of Illinois Urbana-Champaign Veterinary Diagnostic Laboratory.

To calculate DMI during the DP, samples of TMR and pooled refusal for each diet were obtained weekly and dried in an oven to determine DM content. For the lactation phase, the calculation of DMI was different due to the splashing of water on the feed by some cows from their drinking cups. The amounts of feed offered and

refusal were weighed daily for each cow.

Liver biopsy

Liver was sampled via puncture biopsy from cows under local anesthesia at approximately 0900 h. Biopsies were obtained from at least 10 second or greater lactation cows selected randomly from each treatment. Liver tissue samples were frozen immediately in liquid N. A portion of the tissue samples was later analyzed for concentrations of glycogen (Lo, Russell et al. 1970), total lipid (Drackley, Veenhuizen et al. 1991), and triglyceride (Foster and Dunn 1973).

RNA extraction

RNA samples were extracted from frozen tissue using established protocols in our laboratory (Loor et al. 2007). Briefly, liver tissue sample was weighed (~0.3-0.5 g) and straightway was put inside a 15 ml centrifuge tube (Corning Inc. ®, Cat. No. 430052, Corning, NY, USA) with 1 µl of Linear Acrylamine (Ambion® Cat. No. 9520, Austin, TX, USA) as a coprecipitant, and 5 ml of ice-cold Trizol reagent (Invitrogen USA) to proceed with RNA extraction. This extraction procedure also utilizes acid-phenol chloroform (Ambion® Cat. No. 9720, Austin, TX, USA), which removes residual DNA. Any residual genomic DNA was removed from RNA with DNase using RNeasy Mini Kit columns (Qiagen, Hilden, Germany). RNA concentration was measured using a Nano-Drop ND-1000 spectrophotometer (Nano-Drop Technologies). The purity of RNA (A_{260}/A_{280}) for all samples was above 1.81. The quality of RNA was assessed using a 2100 Bioanalyzer, (Agilent Technologies, Santa Clara, CA, USA). A portion of assessed RNA was diluted to 100 ng/µl using DNase-RNase free

water prior to reverse transcriptase.

qPCR analysis

For qPCR, cDNA was synthesized using 100 ng RNA, 1 μg dT18 (Operon Biotechnologies, Huntsville, AL, USA), 1 μL 10 mmol/L dNTP mix (Invitrogen Corp., CA, USA), 1 μL random primer p(dN)6 (Roche® Cat. No. 11034731001, Roche Diagnostics GmbH, Mannheim, Germany), and 10 μL DNase/RNase free water. The mixture was incubated at 65 °C for 5 min and kept on ice for 3 min. A total of 6 μL of master mix composed of 4.5 μL 5X First-Strand Buffer, 1 μL 0.1 M dithiothreitol, 0.25 μL (50 U) of SuperScript™ III RT (Invitrogen Corp.CA, USA), and 0.25 μL of RNase Inhibitor (10 U; Promega, Madison, WI, USA) was added. The reaction was performed in an Eppendorf Mastercycler® Gradient using the following temperature program: 25 ℃ for 5 min, 50 ℃ for 60 min and 70 ℃ for 15 min. cDNA was then diluted 1:4 (v:v) with DNase/RNase free water.

Quantitative PCR (qPCR) was performed using 4 μL diluted cDNA (dilution 1:4) combined with 6 μL of a mixture composed of 5 μL 1 × SYBR Green master mix (Applied Biosystems, CA, USA), 0.4 μL each of 10 μM forward and reverse primers, and 0.2 μL DNase/RNase free water in a MicroAmp™ Optical 384-Well Reaction Plate (Applied Biosystems, CA, USA). Each sample was run in triplicate and a 6 point relative standard curve plus the non-template control (NTC) were used (User Bulletin #2, Applied Biosystems, CA, USA). The reactions were performed in an ABI Prism 7900 HT SDS instrument (Applied Biosystems, CA, USA) using the following conditions: 2 min at 50 ℃, 10 min at 95 ℃, 40 cycles of 15 s at 95 ℃ (denaturation)

and 1 min at 60 ℃ (annealing and extension). The presence of a single PCR product was verified by the dissociation protocol using incremental temperatures to 95 ℃ for 15 s plus 65 ℃ for 15 s. Data were calculated with the 7900 HT Sequence Detection Systems Software (version 2.2.1, Applied Biosystems, CA, USA). The final data were normalized using the genomic mean of six internal control genes: SHPRH, EIF3K, UXT, MRPL39, ACTB and EDC4.

Genes selected for transcript profiling in this study were *ACOX1, APOA1, APOB, ATP5G1, CPT1A, FADS2, FBP1, FOXA2, GHR, GSTM1, HMGCR, HNF4A, HP, IGF1, IL6, NR4A1, PC, PCK1, SCD, SREBF2* and *TNF* (Table 1.1). Primers were designed using Primer Express 2.0 or 3.0 with minimum amplicon size of 80 bp (when possible amplicons of 100-150 bp were chosen) and limited 3' G+C (Applied Biosystems, CA). When possible, primers were designed to fall across exon–exon junctions. Primers were aligned against publicly available databases using BLASTN at NCBI and UCSC's Cow (Bos taurus) Genome Browser Gateway (Table 1.1). Prior to qPCR, primers were tested in a 20 μL PCR reaction using the same protocol described for qPCR except for the final dissociation protocol. For primer testing we used a universal reference cDNA (RNA mixture from 5 different bovine tissues) to ensure identification of desired genes. 5 μL of the PCR product were run in a 2% agarose gel stained with ethidium bromide (2 μL). The remaining 15 μL were cleaned using QIAquick® PCR Purification Kit (QIAGEN) and sequenced at the Core DNA Sequencing Facility of the Roy J. Carver Biotechnology Center at the University of Illinois, Urbana-Champaign. Only those primers that did not present primer-dimer, a

single band at the expected size in the gel, and had the right amplification product (verified by sequencing) were used for qPCR. The accuracy of a primer pair also was evaluated by the presence of a unique peak during the dissociation step at the end of qPCR.

Efficiency of PCR amplification for each gene was calculated using the standard curve method [$E = 10^{(-1/slope)}$] (Table 1.3). Relative mRNA abundance among measured genes was calculated as previously reported (Bionaz and Loor 2008), using the inverse of PCR efficiency raised to Ct (gene abundance = $1/E^{\Delta C}$, where Δ Ct = Ct sample - geometric mean Ct of 3 internal control genes; Table 1.3). Overall mRNA abundance for each gene among all samples measured was calculated using the median Δ Ct. Use of this technique for estimating relative mRNA abundance among genes was necessary because relative mRNA quantification was performed using a standard curve (made from a mixture of RNA) which precluded a direct comparison among genes. Together, use of Ct values corrected for the efficiency of amplification plus internal control genes as baseline overcome this limitation. The overall % relative mRNA abundance is shown in Figure 1.1.

Statistical analysis

The MIXED procedure of SAS (SAS Institute, Inc., Cary, NC, USA) was used for statistical analysis. The fixed effects included diet (CON or OVER), monensin (M or not), time (-14 and 7d relative to parturition), and their interactions. The covariate structure used was AR (1). Data were normalized by logarithmic transformation prior to statistical analysis. All means were compared using the PDIFF statement of SAS

(SAS Institute, Inc., Cary, NC, USA). Statistical significance was declared at $P < 0.05$.

CHAPTER 4

RESULTS

Liver composition

Hepatic concentration of total lipid and triacylglycerol was significantly ($P<0.01$) greater in postpartum than prepartum. In contrast, hepatic glycogen concentration was significantly lower in postpartum than prepartum ($P<0.01$). But there were no differences between groups of different feeding strategies. (Table 1.4)

NEFA and BHBA concentrations

Serum concentration of NEFA during the -2 and -1 week relative to parturition was greater for cows fed CON than for cows fed OVER ($P<0.05$) and showed no significant difference among two diets postpartum. However, NEFA concentration was not affected by monensin. The concentration of BHBA was also higher in cows fed CON at -1 week and was lower in cows fed monensin at 2 week postpartum. (Figure 1.2)

DMI

The DMI prepartum did not differ statistically in the close-up period ($P=0.1$) (Figure 1.4) but had an increase during two weeks after calving ($P<0.05$) (Table 1.6). The DMI was not affected by diet or monensin supplementation. (Figure 1.5)

Fatty acid oxidation and ATP synthesis genes

Among the genes involved in fatty acid oxidation and ATP synthesis, there was an overall increase ($P<0.05$) in the expression of *ATP5G1* and *CPT1A* in all groups

between -14 and 7d. But cows fed CON showed no difference in the expression of *ACOX1* in the same period, whereas OVER still had an increase ($P<0.05$) in the expression. Cows fed CON had a greater expression ($P<0.05$) of *CPT1A* on -14d. In contrast, cows fed OVER had a greater expression of *ATP5G1* on -14d. Both genes did not differ in expression between treatments on 7d. The expression of *ACOX1* was not affected by either of the treatments or their interactions. (Figure 1.6)

Lipid metabolism genes

For genes involved in lipid metabolism, although *APOB* expression was not affected by either of the treatments and interactions, *APOB* expression decreased ($P<0.05$) between -14 and 7d. The expression of *APOA1* was affected by interaction of diet and time on -14d when cows fed CON diet had greater expression than OVER. Also, *APOA1* expression had an increase ($P<0.05$) between -14 and 7d. There was a overall decrease ($P<0.05$) in *SCD* expression between -14d and 7d. Cows fed the CON diet also had a tendency for a lower *SCD* expression relative to the OVER diet on -14d. Besides, cows fed M had higher (monensin × time<0.05) expression than the control group. There was no effect of diet or monensin or their interactions on the expression of *FADS2* and *HMGCR*. *FADS2* expression decreased ($P<0.05$) while *HMGCR* increased between -14 and 7d. (Figure 1.7)

Gluconeogenesis genes

For the genes involved in gluconeogenesis, there was an overall increase ($P<0.05$) in the expression of *FBP1*, *PCK1* and *PC*, regardless of diet and monensin between -14 to 7d. Neither of the treatment nor their interaction had a significant effect on

expression of *FBP1*. There was an interaction effect (P<0.05) for the expression of *PC* and *PCK1* on -14d when it was greater in cows fed CON than OVER. *PC* also had a tendency for a decrease in expression (P=0.1) on -14d in the cows fed monensin. There was an interaction effect (diet × monensin<0.05) in *PC* expression around parturition with CON-M being higher than OVER-M. (Figure 1.8)

Inflammation, acute phase response and oxidative stress genes

For genes involved in inflammation, acute phase response and oxidative stress, the expression of *HP, IL6* and *TNFA* increased between -14 and 7d, whereas the expression of *GSTM1* decreased in the same period. The expression of *HP* and *TNFA* was not affected by diet, monensin or their interaction. However, there was a diet effect and diet x time effect for the expression of *IL6*. On 7 d, cows fed the CON diet had lower (diet × time <0.05) expression of IL6 relative to OVER diet. For *GSTM1*, there was a tendency for a higher (monensin × time<0.1) expression in cows fed M than control group. There was also a tendency for an interaction effect of diet x monensin (P<0.1). (Figure 1.9)

Growth hormone/IGF-1 axis

For genes involved in growth hormone/IGF-1 axis, there was a significant decrease (P<0.05) in the expression of *GHR* and *IGF1* between -14d and 7d. There was a lower (diet × time <0.05) expression of *GHR* in cows fed the CON diet on -14d and a tendency for lower expression in cows fed same diet on 7d. For *IGF1*, cows fed M had a higher (monensin × time <0.05) expression on -14d and cows fed CON diet had a tendency for lower (diet × time <0.1) expression on 7d. (Figure 2.0)

Transcription factors

For genes of transcription factors, overall, there was no time effect on expression of *SREBF2, HNF4A* and *FOXA2*, except *NR4A1* ($P<0.05$). There were also no significant effects of diet and monensin or their interactions on these genes. (Figure 2.1)

CHAPTER 5

DISCUSSION

Blood and liver metabolites

Concentrations of NEFA and BHBA were measured in blood serum to assess energy status. The serum concentration of NEFA during the -2 and -1 week was greater for cows fed CON than for cows fed OVER, but was not affected by M. Moderate increases in NEFA concentration prepartum have been observed previously in cows fed controlled-energy diets relative to those fed higher-energy diets (Janovick, Boisclair et al. 2011). Janovick (2011) also observed the same lower concentration of NEFA postpartum in cows fed controlled-energy diet. In addition, BHBA concentration in -1 week was higher in cows fed CON which might be due to higher concentration of NEFA during that period than that of cows fed OVER.

In our study, M supplementation had no effect on NEFA concentration, different from a previous study (Duffield, Rabiee et al. 2008) which reported that M reduced the serum concentrations of NEFA by 7%. But our observation that BHBA concentration was significantly reduced by M supplementation at -1 week was consistent with Duffield et al. (2008) in which BHBA concentration was reduced by 13% with M supplementation. However, the M effect may be dependent on other factors, such as diet, method of M administration, or stage of lactation. For example, top-dressing with M or using controlled-release capsules (CRC) reduced the effect on BHBA (Duffield, Rabiee et al. 2008). Therefore, the lack of M effect in our study may

be caused by some other factors.

From preaprtum to postpartum, hepatic concentrations of total lipid and triacylglycerol increased significantly ($P<0.05$) whereas glycogen concentration in the liver decreased, consistent with general metabolic and physiological changes in transition cows, which indicated increased fat mobilization, liver uptake of NEFA, and increased demand for glucose for lactose synthesis. There were no differences in the concentrations of these liver metabolites within diets and use of M.

DMI

No significant difference was observed in dry matter intake (DMI) between diets and the use of monensin during the close-up period and two weeks after calving. The DMI increased after calving which was consistent with many previous studies on DMI change during transition period (Bertics, Grummer et al. 1992). However, DMI from any group in our trial did not have a decline before calving which was inconsistent with previous studies. Regulation of DMI in periparturient cows is complex and far from understood (Grummer, Mashek et al. 2004), but it is presumed that high circulating estrogen may be one factor that contributes to decreased DMI around parturition (Grummer 1993). As demonstrated in previous study (Martineau, Benchaar et al. 2007), feeding monensin usually does not affect DMI. Our study also revealed a lack of monensin effect on prepartum or postpartum DMI.

Fatty acid oxidation and ATP synthesis genes

Entry of NEFA into the mitochondria for beta-oxidation to carbon dioxide or ketone bodies is controlled by the enzyme carnitine palmitoyltransferase (CPT-1)

encoded by *CPT1A* in liver. Activity of CPT-1 is inhibited by malonyl-CoA. An increase of mRNA abundance for CPT1A may reflect the increase in oxidation of fatty acids because of an elevated level of NEFA during negative energy balance (Graber, Kohler et al. 2010). Another study showed that hepatic mRNA expression for *CPT1A* was positively associated with both liver TAG and serum BHBA and NEFA concentrations in periparturient dairy cows (Loor, Dann et al. 2005). Our observation of increased expression of *CPT1A* after calving was obviously correlated with fat mobilization from adipose tissue and elevated uptake of liver for NEFA during transition period. In Litherland et al. (2011) study, their data highlighted the strong relationships between serum NEFA concentrations on the day of parturition and d 1 postpartum with the degree of hepatic lipid accumulation in dairy cows. They also found that controlled energy intake during the dry period seems to direct palmitate toward oxidation and away from esterification.

Our data revealed a positive effect of the CON diet on *CPT1A* mRNA expression at -14d, which may be due to the higher serum NEFA in those cows. Similar findings showed that hepatic *CPT1A* transcript abundance and CPT-1 activity was increased by moderate nutrient restriction during the dry period (Loor, Dann et al. 2006). Another study with mid-lactation cows also reported higher expression of *CPT1A* by feed restriction (Akbar 2013). However, increased *CPT1A* expression does not necessarily indicate an elevated fatty acid oxidation. The liver increases its capacity to metabolize fatty acids through increasing substrates or change in expression of some key molecules such as CPT-1 rather than through greater expression of metabolic

pathways (Bionaz and Loor 2012).

Fatty acid oxidation can occur in peroxisomes alternatively, serving as adaptation of FA metabolism in liver during the periparturient period (Grum, Drackley et al. 2002). Increases in hepatic capacity for peroxisomal fatty acid oxidation may help the liver cope with large influx of mobilized NEFA and help to prevent excessive accumulation of fat in the liver. *ACOX1*, which encodes acyl-CoA oxidase, the flux-generating enzyme of peroxisomal beta-oxidation of fatty acids, increased after parturition (Loor, Dann et al. 2005). Similarly, in the present study, we observed a general increase in expression of *ACOX1* after calving. Increased expression of *CPT1A* and *ACOX1* after calving was likely due to activation of PPARα by increased circulating NEFA (Loor, Dann et al. 2005). The activation of PPARα increased palmitate oxidation in liver of dairy calves along with an increase in expression of several genes known to be PPARα targets involved in FA oxidation including *CPT1A* (Litherland, Bionaz et al. 2010). It was also demonstrated that LCFA, provided from NEFA originating from adipose tissue lipolysis, could activate PPARα and regulate downstream gene expression in bovine cells (Bionaz, Chen et al. 2013).

Mitochondrial ATP synthase, encoded by *ATP5G1* gene, catalyzes ATP synthesis, utilizing an electrochemical gradient of protons across the inner membrane during oxidative phosphorylation. Increased hepatic expression of *ATP5G1* postpartum indicated a higher requirement for energy in cows after calving and therefore up-regulated oxidative phosphorylation. Our data showed that the cows fed the OVER diet had higher expression of *ATP5G1* than cows fed the CON at -14d because the

CON diet led to decreased supply of propionate, and then decreased glucose production. Data from another study revealed that decreased gluconeogenesis in food-restricted cows might be associated with lower expression of genes coding for proteins in the oxidative phosphorylation pathway and they found over 80% of genes with some function in oxidative phosphorylation including *ATP5G1* and mitochondrial electron transport were down-regulated (Loor, Everts et al. 2007). Similar results were presented in another study as well (Akbar 2013).

Lipid metabolism genes

Apolipoprotein A-I (apoA-I) encoded by *APOA1* and Apolipoprotein B (apoB) encoded by *APOB* are two important components of circulating lipoproteins and play a critical role in cholesterol transport and lipoprotein synthesis, serving as important biomarkers of ketosis and associated problems in transition cows (Bobe, Young et al. 2004). It has been well recognized that peripartal blood cholesterol concentration is one important predictor of liver function and overall health and productivity of the cow (Bertoni, Trevisi et al. 2008). APOB is necessary for the formation of TAG-rich VLDL particles in liver that are subsequently exported to peripheral tissues and APOA1 is needed for HDL synthesis which carries cholesterol from peripheral tissues to liver. Therefore, it has been speculated that decreased serum concentrations of APOB, APOA1 and LCAT, which requires apoA-I for activation (Williamson, Lee et al. 1992), are intimately related to development of fatty liver and ketosis.

In the present study, the expression of *APOA1* was higher ($P<0.05$) in cows fed CON than OVER at -14 d, which was similar to the result of up-regulated expression

of *APOA1* in feed-restriction-induced ketotic cows (Loor, Everts et al. 2007) and down-regulated expression in cows overfed pre partum (Loor 2010). *APOB* was also up-regulated in that study (Loor, Everts et al. 2007), which was contrary to our results of no diet effect on *APOB* expression. Decreased concentrations of apoprotein B and apoprotein A-I, along with decreased formation of lipoproteins, were highly correlated with development of nonalcoholic fatty liver disease (NAFLD) (Karavia, Papachristou et al. 2012; Jiang, Robson et al. 2013). Therefore, we speculate the higher expression of *APOA1* appears to indicate a better hepatic function in lipid metabolism. The fact that up-regulation of *APOA1* and *APOB* along with limited VLDL synthesis and export during ketosis suggested other mechanisms led to liver lipidosis (Loor, Everts et al. 2007).

Monensin supplementation appeared to have a lowering effect ($P<0.05$) on the expression of *APOA1* at -14 d. This effect seemed to be negative for cows in the adaptation to negative energy balance during transition period.

Fatty acid desaturase 2 (FADS2) and Stearoyl-CoA desaturase (SCD) are two critical enzymes for fatty acid desaturation. Both genes experienced an overall decrease in expression after parturition. Previous studies also found a 5-fold decrease in *SCD* mRNA between -14 d and 1 d postpartum in cows (Bionaz 2007). Transcription of *SCD* was speculated to play a role in providing endogenous oleic acid for TAG synthesis and VLDL secretion (Loor, Everts et al. 2007). Mice with a targeted disruption in *SCD* (Scd-/-) have significant reductions in TAG concentrations in tissues, suggesting that monounsaturated fatty acids synthesized endogenously are

important for normal TAG synthesis (Martineau, Benchaar et al. 2007).

A tendency for a diet effect on expression of *SCD* showed that the CON diet appeared to down-regulate *SCD* expression, which was consistent with previous results in cows with feed-restricted-induced ketosis (Loor, Everts et al. 2007). Down-regulation of *SCD* is suggestive of impaired de novo synthesis of oleic acid, which is the major monounsaturated fatty acid in cellular membranes. Therefore, VLDL synthesis and secretion from liver was impaired as endogenous synthesis of oleic acid is required for VLDL synthesis, therefore leading to liver lipidosis. However, sustained down-regulation of *SCD* expression in mice prevents diet-induced hepatic insulin resistance, obesity, and liver lipidosis (Flowers, Rabaglia et al. 2007). Monensin supplementation also had a significant down-regulating effect on *SCD* expression at -14 d. But neither diet nor monensin supplementation effect in present study was observed after parturition.

Gluconeogenesis genes

PC, *PCK1* and *FBP1* code for key regulatory enzymes in the gluconeogenic pathway. Pyruvate carboxylase (PC) and cytosolic phosphoenolpyruvate carboxykinase 1 (PCK1) are potential rate-limiting enzymes for hepatic gluconeogenesis (Greenfield, Cecava et al. 2000). PC converts pyruvate to oxaloacetate and PCK1 catalyzes the conversion of oxaloacetate to phosphoenolpyruvate. The elevated mRNA expression of these three genes from prepartum to postpartum reflected the adaptation of liver to the increased requirement of glucose in early lactation, denoting an attempt to increase hepatic gluconeogenesis

from propionate, lactate and amino acids, similar to previous findings (Greenfield, Cecava et al. 2000; Karcher, Pickett et al. 2007).

In our study, expression of *PC* and *PCK1* were very similar, both of which were affected by diet at -14d with higher expression in CON group. This pattern was similar to previous findings which showed expression of PC was up-regulated by feed restriction (Loor, Everts et al. 2007), however, in that study, *FBP1* was also up-regulated. Higher expression of *PC* and *PCK1* was likely associated with the need to maintain blood glucose concentrations in the cows fed CON diet. Similar results have been showed in an early study in which fasting led to higher expression of gluconeogenesis genes in rodents(Lemaigre and Rousseau 1994). On the contrary, in OVER group, a greater influx of propionate, greater hepatic glucose output and hyperinsulinemia could have affected PC expression through a feedback mechanism.

On the contrary, in the OVER group, a greater influx of propionate, greater hepatic glucose output and hyperinsulinemia could have affected *PC* expression through a feedback mechanism (Velez and Donkin 2005). In the Velez and Donkin (2005) study, higher PC mRNA with unchanged *PCK1* expression may have been coupled to increased protein turnover during feed restriction and enhanced metabolism of lactate, alanine, and other amino acids.

Recent findings indicate that the promoter 1 region on the *PC* gene in glucogenic tissues may be responsive to the presence of NEFA in food-restricted cows (White, Koser et al. 2011). In the current study, elevated expression of *PC* was also parallel to elevated plasma NEFA concentration in cows fed CON diet. Besides, gluconeogenesis

was believed to be mediated by some hormones. Insulin suppresses gluconeogenesis by reducing the transcription rate of *PCK1* gene (Zhang, Chen et al. 2011), and combined with the elevated NEFA concentration during the same period, which appeared to be negatively associated with insulin level (Ramos-Roman, Lapidot et al. 2012), the higher expression of *PCK1* likely indicated a lower serum insulin level in cows fed CON diet.

We observed a tendency of higher expression of *PC* in cows fed with monensin at -14d, contrary to another study in which *PCK1* was up-regulated by monensin while *PC* was not affected at the same day (Karcher, Pickett et al. 2007). Despite that prepartum gluconeogenesis genes expression was altered by close-up diet, this effect did not persist after calving, reflecting the limit of close-up diet effect on transcription of these genes.

Inflammation, acute phase response and oxidative stress genes

There is believed to be a causal link between hepatic inflammation and liver TAG accumulation (Loor, Dann et al. 2005). Our data showed genomic adaptations of genes related to inflammation, acute phase response, and detoxification in response to different treatments. Overall, the CON diet caused a lower effect on *IL6* expression, suggestive of nutrition playing a role in bovine hepatic inflammation. IL6 serves as a biomarker particularly for hepatic disease and inflammation as it has been widely studied in non-ruminants (Moshage 1997). It has been demonstrated that increased hepatic mRNA expression of pro-inflammatory cytokines (e.g. TNFA, IL6) was correlated to liver TAG accumulation and elevated serum NEFA in mice fed high-fat

diets (Cai, Yuan et al. 2005). Therefore, the lower expression of *IL6* likely revealed an attenuated inflammation status in cows fed CON diet.

Another study showed a remarkable increase of *IL6* expression in cows with ketosis (Loor, Everts et al. 2007). Several previous studies also showed the important role of IL6 in hepatic functions in dairy cow such as fatty acid oxidation (Kagawa, Azuma et al. 1999), lipoprotein metabolism (Gervois, Kleemann et al. 2004) and transcription regulation (Horton, Shah et al. 2003), therefore making IL6 as an indicator of impairment in liver functions. It remains to be determined whether *IL6* expression only serves as a general response of liver to metabolic disturbance in transition cows or it has other regulation functions in onset of some metabolic disorders.

In our study, expression of another important pro-inflammatory gene *TNFA* was not affected by diet. The increase of *TNFA* between -14d to 7d in present study was consistent to previous study in which expression of *TNFA* increased as parturition approaches (Loor, Dann et al. 2005), and was believed to be associated with increased NEFA and decreased feed intake in another study (Kushibiki, Hodate et al. 2003).

An overall decreased expression of *GSTM1* which encodes glutathione S-transferase that belongs to the mu class, indicated an impaired detoxification capacity in liver. Down-regulated *GSTM5* gene (another gene encoding the enzyme of same class) suggests that the liver from the cows would have been at higher risk of pathogenic insults as well as oxidative stress damage (Loor 2010). In cows fed to meet prepartal energy requirements, expression of *GSTM5* decreased by 1 day

postpartum and remained down-regulated through the first 2 weeks postpartum (Loor, Dann et al. 2005), a period that is characterized by increased oxidative stress (Bernabucci U 2005). Supplementation of monensin likely had a beneficial effect of up-regulating *GSTM1* expression at -14 d in present study, suggesting more capacity than control group to detoxify electrophilic compounds, including products of oxidative stress.

Growth hormone and IGF-1 axis

Growth hormone/IGF-1 axis plays a pivotal role in adaptation mechanisms of transition cows, especially in hepatic glucose and lipid metabolism. IGF-1 is a primary mediator of the effects of GH and GH can stimulate IGF-1 generation. Plasma IGF-1 concentration was reported to be closely related to the insulin status of cows (Butler, Marr et al. 2003). During NEB in transition period, the decline in circulating IGF-1 around parturition is paralleled by a decline in plasma insulin. Changes in plasma GH over the same period are opposite to that of IGF-1 and insulin (Bell, Burhans et al. 2000). GH receptors (GHRs) are found in many tissues and the liver is the site of greatest abundance (Lucy, Boyd et al. 1998). It has been well established that hepatic GHR concentration is also positively correlated with plasma IGF-1 and the level of nutrition (Breier 1999).

GHR and *IGF1* expression experienced a decrease after parturition (Gross, van Dorland et al. 2011), as confirmed in the present study. Early lactation in dairy cows is characterized by a hypoinsulinemic state, which favors an increased oxidation of fatty acid and decreased glucose oxidation. It was found that lower serum insulin

concentration down-regulated hepatic *GHR* expression and *IGF1* expression (Butler, Marr et al. 2003). In the present study, *GHR* expression was lower in cows fed CON diet at two time points, which can be explained that CON diet led to lower insulin concentration and further down-regulate *GHR* expression. The *IGF1* expression also had a tendency in down-regulation effect of CON diet at 7 d. Moreover, other study showed that gluconeogenesis genes *PC* and *PEPCK* were decreased by IGF-1 treatment, which was in agreement with our results that lower expression of *IGF1* was parallel with higher expression of *PC* and *PCK1* in cows fed CON (Wang, Zhu et al. 2012). Therefore, the pattern of lower expression in both genes seemed to indicate a beneficial state for cows in which oxidation of fatty acid increased and glucose oxidation decreased, sparing glucose supply for milk synthesis in mammary gland.

Transcription factors

Many studies have investigated the roles of several transcription factors in liver metabolism in dairy cows. PPAR-alpha was reported to mediate effects of NEFA on peroxisomal and mitochondrial fatty acid oxidation and up-regulates genes associated with ketogenesis and play a central role in these metabolic events in periparturient bovine liver (Loor, Dann et al. 2005). *PPARA* upregulation also has anti-inflammatory responses by inhibiting expression of proinflammatory ctykines and acute-phase proteins (Mandard, Muller et al. 2004). Another important transcription factor is HNF4A which is known to have an important role in fatty acid oxidation and gluconeogenesis through binding of the promoter region of *ACOX1* and *PCK1* (Odom, Zizlsperger et al. 2004) and it is mediated by periparturient serum NEFA

concentrations.

However, in the present study, we did not observe any effect of treatments on mRNA expression of four transcription factors, which was consistent with the lack of treatment effects on majority of other genes.

CHAPTER 6

CONCLUSIONS

Results in the present study revealed the hepatic adaptation and response in transition cows in terms of gene expression to four different prepartum feeding strategies: one-stage controlled energy-high fiber diet (CON) fed throughout the entire dry period and two-stage with the same CON fed in far-off dry period followed by a higher energy diet in close-up period (OVER) with or without inclusion of monensin. Diet effects on the expression of few genes were primarily observed at -14 d. Inclusion of monensin appeared to have even less effect on these genes. Along with data from DMI, blood and liver metabolites, we concluded that two-stage diet (OVER) had no advantage compared with the single-group controlled energy-high fiber diet (CON) and inclusion of monensin had very little benefit in improving hepatic adaptations to metabolic changes in transition cows.

CHAPTER 7

TABLES AND FIGURES

Table 1.1. GenBank accession number, hybridization position, sequence and amplicon size of primers used in this study.

Accession #	Genes	Primers[1]	Primers (5'-3')[2]	bp[3]
NM_001083772.1	*GSTM1*	F.604	CACATGGCCAGGATCTGTTACA	100
		R.703	CCAGAAAATCTGAGAAGAGCTTCAT	
XM_002694902.1	*EDC4*	F.1237	GCGGAAGGTCCTCTACGTGAT	104
		R.1340	AGCTCAGTACAGGGTGGGTTAGAA	
XM_002690300.1	*SHPRH*	F.1985	CAATCAAGAACATTCAGCAAAGGA	100
		R.2084	ACTGGAAGAATTATAGGAACCCATGA	
NM_001034489.1	*EIF3K*	F.174	GGAGACACAGGCCAAAGAAAAT	100
		R.273	GGTGACCGTGGTCTGGAAGA	
FJ415874.1	*CPT1A*	F.141	TCGCGATGGACTTGCTGTATA	100
		R.240	CGGTCCAGTTTGCGTCTGTA	
BC102952.1	*ATP5G1*	F.394	CTATGCCAGGAACCCGTCTCT	101
		R.494	AGGCGACCATCAAACAGAAGA	
NM_001083444.1	*FADS2*	F.142	ATCGATCGCAAGGTCTACAACA	102
		R.243	GAAGGCGTCCGTAGCATCTT	
NM_001015557.1	*HNF4A*	F.316	ACGACCAGGTGGCCCTACTC	120
		R.435	GCCGAGGGACGATGTAGTCA	
NM_177946.3	*PC*	F.3577	GCAAGGTCCACGTGACTAAGG	124
		R.3700	GGCAGCACAGTGTCCTGAAG	
NM_001035289.2	*ACOX1*	F.180	ACCCAGACTTCCAGCATGAGA	100
		R.279	TTCCTCATCTTCTGCACCATGA	
XM_002691511.1	*APOB*	F.2232	CAAGGCTTTGTACTGGGTTAACG	105
		R.2336	ACCATGTCCTGCTCATGTTTATCA	
XM_599153.4	*FOXA2*	F.1924	AATAATAATGTAAGGGACTGTTGTAAATGC	120
		R.2043	AAAGCACGCAGAAACCATAAATTAGT	
NM_176608.1	*GHR*	F.1350	CTAGCCAGCAGCCCAGTGTTA	110
		R.1459	TGGATTGCTGAGCTGTGTATGG	
NM_001077828.1	*IGF1*	F.166	CCAATTCATTTCCAGACTTTGCA	103
		R.268	CACCTGCTTCAAGAAATCACAAAA	
EU276071.1	*IL6*	F.190	CCAGAGAAAACCGAAGCTCTCAT	100
		R.289	CCTTGCTGCTTTCACACTCATC	

[1] Primer direction (F – forward; R – reverse) and hybridization position on the sequence.

[2] Exon-exon junctions are underlined.

[3] Amplicon size in base pair (bp).

Table 1.1. (continued) GenBank accession number, hybridization position, sequence and amplicon size of primers used in this study.

Accession #	Genes	Primers[1]	Primers (5'-3')[2]	bp[3]
NM_174737.2	*PCK1*	F.601	AAGATTGGCATCGAGCTGACA	120
		R.720	GTGGAGGCACTTGACGAACTC	
XM_002687950.1	*SREBF2*	F.1089	GCACAAGTCTGGCGTTCTGA	112
		R.1200	GCTCTTCTGATTGGCCAGCTT	
NM_001075911.1	*NR4A1*	F.1469	GCAAGTGGGCCGAGAAGA	104
		R.1572	GGCCAGACGGAGGATAAAGAG	
M35870.1	*APOA1*	F.307	ACGTTGTCCAAAGTGCGTGA	101
		R.407	TCCTTGTGCATCTCCTGCCT	
AY141970	*ACTB*	F.624	GCGTGGCTACAGCTTCACC	54
		R.677	TTGATGTCACGGACGATTTC	
NM_001034447	*FBP1*	F.396	GAAGAAGATGAACATGCCATAATAGT	95
		R.490	CAGTCGATGTTGGATGATCCAT	
BE588673	*HMGCR*	F.262	GAGTGGCAGGACCTCTGTGC	121
		R.382	GCACCTCCACCAAGGCCTAT	
BC109668	*HP*	F.1210	GGTTCGGAAAACCATCGCTA	101
		R.1310	CACTCGTGTCCCCTCCACTC	
NM_001080730.1	*MRPL39*	F.493	AGGTTCTCTTTTGTTGGCATCC	101
		R.593	TTGGTCAGAGCCCCAGAAGT	
AY241933	*SCD*	F.665	TCCTGTTCTTCTGCTTCATCC	101
		R.765	GGCATAACGGAATAAGGTGGC	
EU276079	*TNFA*	F.174	TCTCAAGCCTCAAGTAACAAGCC	114
		R.287	CCATGAGGGCATTGGCATAC	
NM_001037471	*UXT*	F.323	TGTGGCCCTTGGATATGGTT	101
		R.423	GGTTGTCGCTGAGCTCTGTG	

[1] Primer direction (F – forward; R – reverse) and hybridization position on the sequence.

[2] Exon-exon junctions are underlined.

[3] Amplicon size in base pair (bp).

Table 1.2. Sequencing results of PCR products from primers of genes designed for this experiment. Best hits using BLASTN (http://www.ncbi.nlm.nih.gov) are shown.

Genes	Sequence
GSTM1	GCCAGTGACGTACCGTGGTACTGAGGAATTCCGTGAAGGATGAAGCTCTTCTC AGATTTTCTGGGAT
EDC4	GCGCTAACGGGAGGAGGGCCGGGGGGCCCGGTCCTTCAGCTCCATCTCGTAG TTTCCTTCTAACCCACCCTGGTACTGAGCTTAAA
SHPRH	CAGTACTCTTACTGTTGTGGTAGTACAGTCCACATTCTAATATCATGGGTTCCTA TAATTCTTCCAGTAA
EIF3K	TGTGACACGTGCGTTCGTAACGTTACCAGTTCACCCGGCCTTCTTCCAGACCA CGGTCACCAA
CPT1A	GCATGGTGTATATCTACCGACTCACACCAGGGCAAGAGCTGGCACGGGATCCC GCCATCCTGCTTTACAGACAACTGG
ATP5G1	GCATCTCTCTCTATGCCATTCTGGGCTTTGCCCTGTCTGAGGCTATGGGGCTCTT CTGTTTGATGGATCGCACTACG
FADS2	CGTCGCAGGTCTACAACATCATAAATGGTCAGCCGCACCCGGGCCAGCGGGCA TCGGCCTATGCGGGAAATGCTACG
HNF4A	AGTGCGCTTGCAGGTCAAGTGCTTCCGGGCTGCATGAAGAGGAAGCTCAGAA TGAACGGAGGATCAGCACTCCCGGTCCAGCTACG
PC	GATCATAGGAGTACAGAACTCATCTGGAAGAATCGAGTGACCACGCTGAGACT GGCAGCCTGACCATCCCCGACCCCTGCCTTCAGGACACTGTGCTGCCAGA
ACOX1	ATCCTCGTATCCGCGTTCAGGGTGCGTTTAAGAAGAGTGCCATCATGGTGCAG AAGATGAGGAAATCCCC
APOB	TGGTCATGGAGTTGTTCAACGGTAAAGACTGAACCAAAGATCCCAGAAGCAG CCTACCTCCGATCTTGGGAGAAGAG
FOXA2	CAGAAGAAGAAAGCATTCGCATTCTTGATACGGGGAAATCCAGGTCTCAAGTC TGACTAATTTATGGTTTCTGCGTGCATTTAA
GHR	TAGCGACCCAGTGTTTTAGTAGGACAAACCAAGACCACCATTGGTGGCGAGTC ACATCAAGCTGCCACACAGCC
IGF1	TCATCTTCCAGTTTGCTAAGCAATGGGAAAAGCAGTCTTCACCCAATTCGTATT TAAGTGCTGCGTTT
IL6	ATCTCTGCAAGAAAGGAGATAGTGAGAGAATGAGTGTAAACAGCAGACACTG GAAAATAAGCTAATCTTCCAAA

Table 1.2. (continued) Sequencing results of PCR products from primers of genes designed for this experiment. Best hits using BLASTN (http://www.ncbi.nlm.nih.gov) are shown.

Genes	Sequence
PCK1	GCCATGTGTACAGCAGTCGCATCATGACGAGGATGGGCACCAGCGTCCTGGA AGCGCTGGGGGACGGCGAGTTCGTCAAGTGCCTCCACAAA
SREBF2	GCATCACTTCAATACTTGCAGCGGTCATCATAACTGCGCCGGGAGAACATGG TGCTGAAGCTGGCCAATCAGAAGAGCAACCAT
NR4A1	GCGTTAGTCTTCGGGAACAGGACCGTCCGGTCGTGAGCCGCCTTTCTGGAGC TCCTTTATCCTCCGTCGCCAA
APOA1	TGTAAAGCGTGAACAGCTGGGCCGTGACCCAGGAGTCGGACAACCTGGAAG GAGAGTCGCTGAGGCGATGC
ACTB	CACTTCTAGCTGCGTGTGGCCCCCTGAGGAGCACCCCGTGCTGCTGACCGAG GCTCTGAACCCCAAGGCCAACAGAAGATCATGTTCA
FBP1	ATGAACATGCATAATAGTGACTGAAGGAATATGTGTTGTGACCCTTGATGGCA C
HMGCR	GGACATCATTTCTGAATAAATCATAGCAATATTGATTTACTTGTTCCAGATACG CCACTTGGATCAAATATTTTGGGTT
HP	GTTGGACCATCGCTACAACTAAGCGGCTCTAGGCAGGCCTGAGGGAAGCCA CAGGAGAGG
MRPL39	TTATTCAAAACAACGTCATAGCAGAAGGCTCCAGCGATTACTGGAACTTCTG GGGCTCTGCA
SCD	AGACTGTGCATGGTATCTGTGGGATGAAACGTTTCAAAACAGCCTGTTTTTT GCCACCTTATTCCGTATATAGCCAG
TNFA	CTGGTTCAACACTCAGGTCCTCTTCTCAAGCCTCAAGTAACAAGCCGGTAGC CCACGCTTGTAGCCGAAA
UXT	CTGGTTATCGTGGAGCTCTCAGTTCATTGATCGTAAGAGCAGTCTCCTCACAG AGCTCAGCGACAATCTCAT

Table 1.3. qPCR performance of genes measured

Gene	Median Ct[1]	Median ΔCt[2]	Slope[3]	(R^2)[4]	Efficiency[5]
ACOX1	20.74	-2.59	-3.53	0.996	1.92
APOA1	20.13	-3.46	-3.45	0.999	1.95
APOB	21.32	-2.03	-3.54	0.999	1.92
ATP5G1	23.88	0.54	-3.45	0.993	1.95
CPT1A	19.85	-3.51	-3.26	0.991	2.03
FADS2	24.19	0.56	-3.08	0.993	2.11
FBP1	21.75	-1.55	-3.56	0.997	1.91
FOXA2	26.50	3.08	-3.8	0.994	1.83
GHR	22.02	-1.57	-3.84	0.991	1.82
GSTM	27.89	4.48	-2.3	0.995	2.72
HMGCR	22.40	-1.14	-2.72	0.994	2.33
HNF4A	19.60	-3.78	-3.73	0.996	1.85
HP	15.66	-7.5	-3.83	0.991	1.82
IGF1	24.58	0.86	-3.45	0.996	1.95
IL6	26.42	2.94	-3.1	0.993	2.10
NR4A1	30.01	6.46	-3.19	0.986	2.06
PC	20.91	-2.48	-4.79	0.994	1.62
PCK1	20.30	-3.22	-3.56	0.997	1.91
SCD	23.33	-0.11	-3.65	0.997	1.88
SREBF2	24.31	0.93	-3.12	0.993	2.09
TNFA1	30.76	7.08	-2.58	0.991	2.44

[1] The median is calculated considering all time points and all steers.

[2] The median of ΔCt is calculated as [Ct gene – geometrical mean of Ct internal controls] for each time point and each steer.

[3] Slope of the standard curve.

[4] R^2 stands for the coefficient of determination of the standard curve.

[5] Efficiency is calculated as $[10^{(-1 / \text{Slope})}]$.

Table 1.4. Composition of liver tissue from cows around parturition

Liver		Time relative to parturition			*P* value						
Composition[a]	Diet	-14	7	SEM	D	M	T	D×M	D×T	M×T	D×M×T
Glycogen	OVER	3.44	1.56	0.76	0.29	0.71	<0.01	0.29	0.95	0.79	0.94
	OVER-M	3.26	1.05								
	CON	2.47	0.45								
	CON-M	3.26	1.06								
Triglyceride	OVER	0.48	5.43	0.95	0.37	0.93	<0.01	0.64	0.58	0.31	0.73
	OVER-M	0.43	6.08								
	CON	1.68	5.71								
	CON-M	0.78	6.20								
Total lipid	OVER	4.21	9.72	2.17	0.33	0.75	<0.01	0.78	0.66	0.32	0.51
	OVER-M	3.84	10.17								
	CON	5.32	10.29								
	CON-M	4.06	12.98								

[a] Unit is % wet weight of liver tissue biopsied at -14 and 7 d.

Table 1.5. Serum concentrations of non-esterified fatty acids (NEFA) and beta-hydroxybutyric acid (BHBA) from cows around parturition

Blood Metabolites	Treatments	Weeks relative to parturition														*P* value						
		-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	SEM	D	M	T	D×M	D×T	M×T	D×T×M
NEFA	OVER	0.42	0.41	0.35	0.33	0.50	1.24	1.01	0.83	0.71	0.66	0.68	0.71	0.59	0.13	0.86	0.36	<0.01	0.37	<0.01	0.46	0.21
m*M*	OVER-M	0.50	0.45	0.44	0.45	0.58	1.06	0.92	0.86	0.82	0.70	0.74	0.66	0.45								
	CON	0.35	0.37	0.49	0.54	0.73	0.99	0.82	0.71	0.55	0.56	0.41	0.56	0.41								
	CON-M	0.48	0.50	0.49	0.59	0.89	1.14	1.00	0.76	0.88	0.69	0.66	0.71	0.54								
BHBA	OVER	0.40	0.41	0.43	0.43	0.51	0.99	1.38	0.93	0.91	0.83	0.68	0.60	0.58	0.22	0.65	0.49	<0.01	0.23	0.35	<0.01	0.95
m*M*	OVER-M	0.41	0.42	0.41	0.42	0.45	0.67	0.69	0.64	0.61	0.62	0.49	0.52	0.43								
	CON	0.39	0.38	0.44	0.49	0.63	1.15	1.13	0.69	0.71	0.54	0.55	0.56	0.51								
	CON-M	0.48	0.49	0.45	0.62	0.83	1.01	0.88	0.72	0.77	0.98	0.58	0.51	0.52								

Table 1.6. Postpartum dry matter intake of cows

	Treatments	Weeks relative to parturition			*P* value						
		1	2	SEM	D	M	T	DxM	DxT	MxT	DxTxM
DMI, kg	OVER	10.89	13.75	1.77	0.77	0.16	<0.01	0.26	0.74	<0.05[a]	0.25
	OVER-M	14.53	18.33								
	CON	12.93	14.44								
	CON-M	11.86	16.43								

[a] Interaction effect of monensin and time (P<0.05).

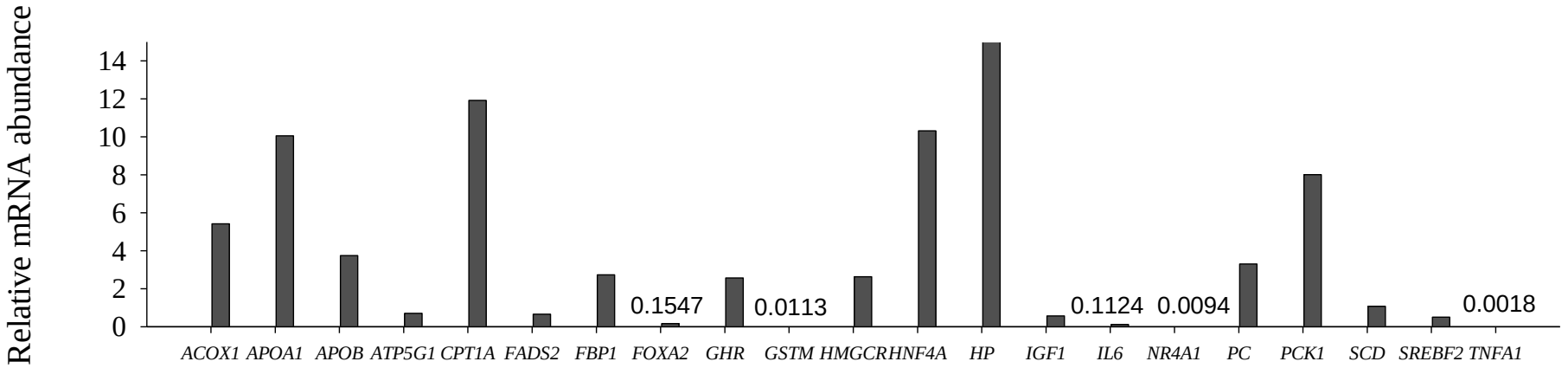

Figure 1.1. mRNA abundance of genes measured in this study

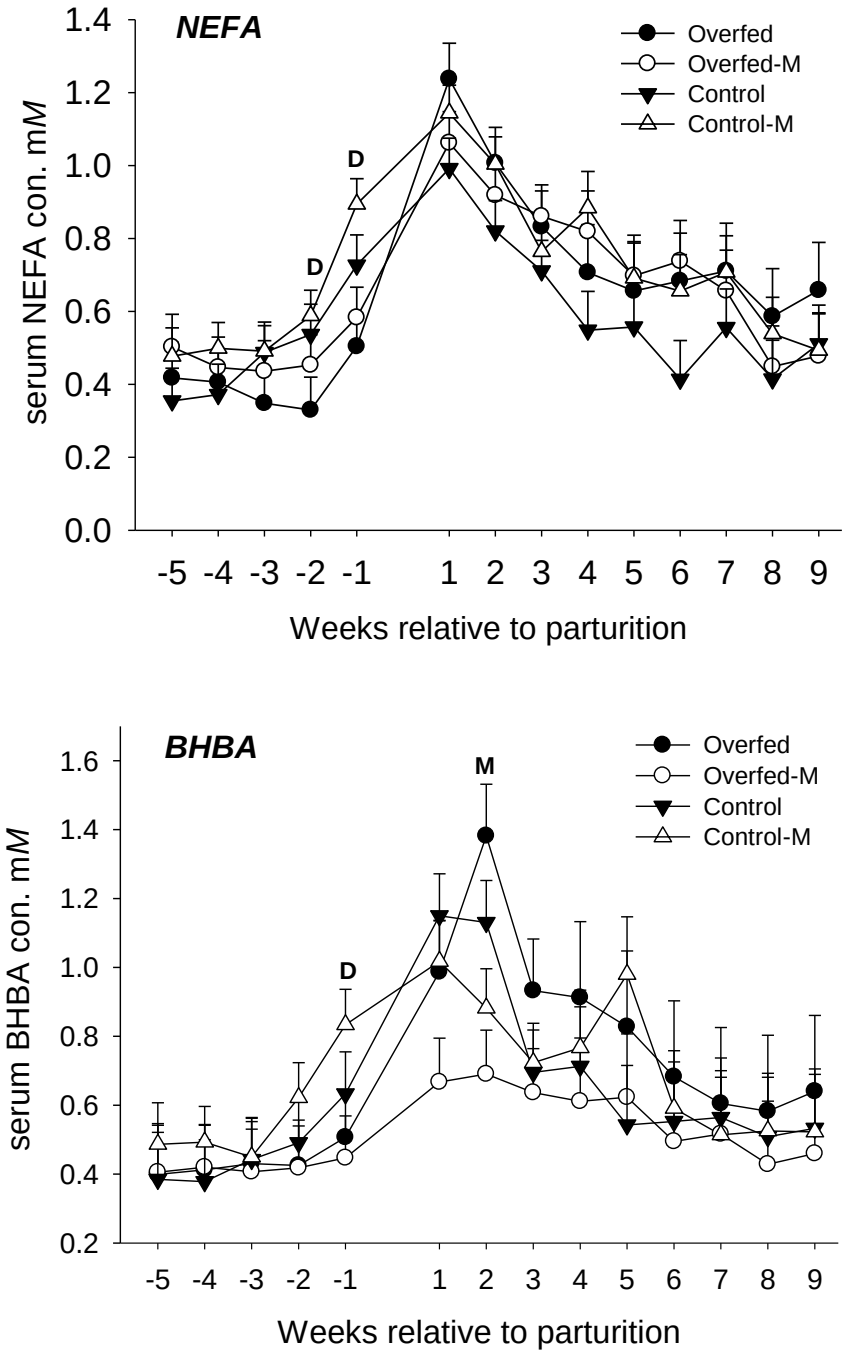

Figure 1.2. Mean weekly serum concentration of non-esterified fatty acids (NEFA) and beta-hydroxybutyric acid (BHBA) in cows around parturition. D indicates an interaction effect of diet x time ($P<0.05$) at the specific time point. M indicates an interaction effect of monensin x time ($P<0.05$) at the specific time point.

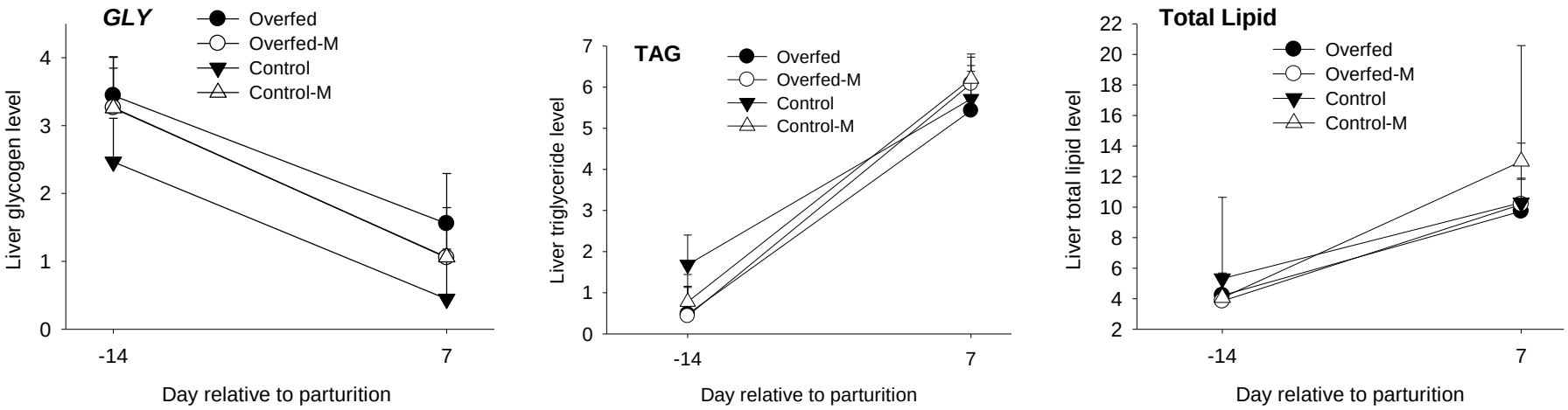

Figure 1.3. Composition of liver tissue from cows in the experiment. GLY=Glycogen; TAG= Triacylglycerol; and Total Lipid. They were calculated as % wet weight of liver tissue biopsied at -14 d and 7 d.

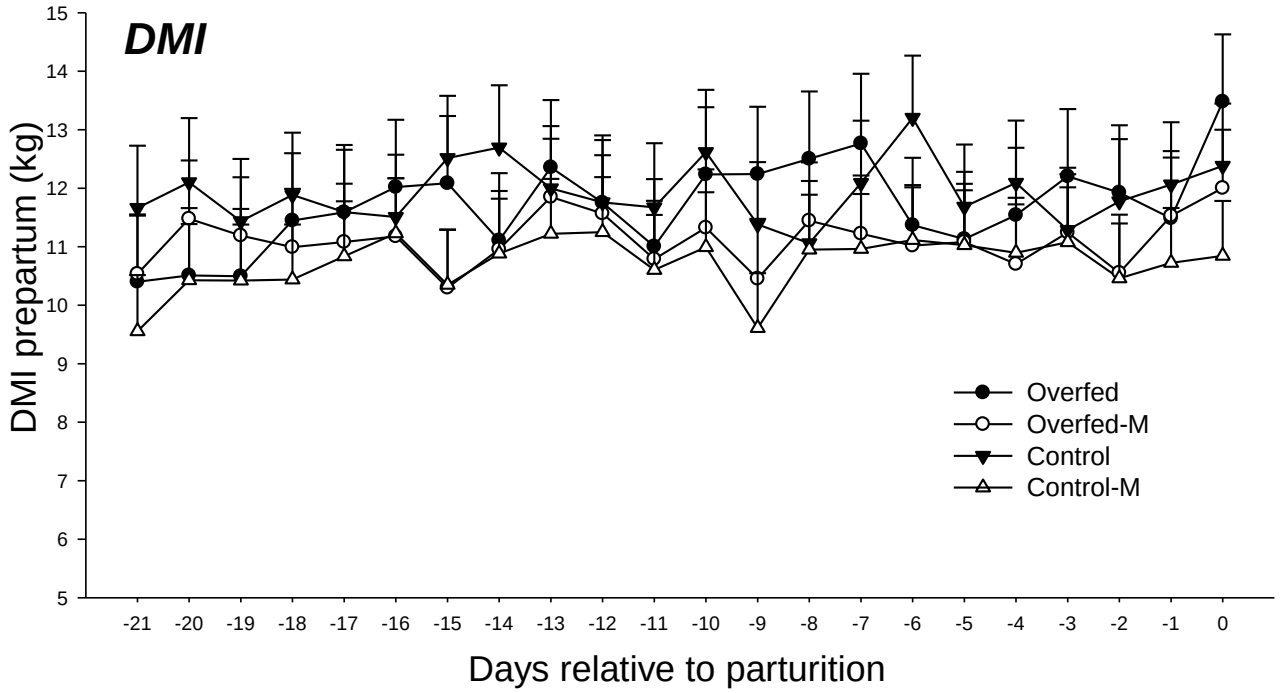

Figure 1.4. Mean daily dry matter intake (DMI) in cows before parturition. Effects in model: time (*P*=0.10), diet (*P*=0.92), monensin (*P*=0.30), diet x monensin (*P*=0.72), diet x time (*P*=0.82), monensin x time (*P*=0.90).

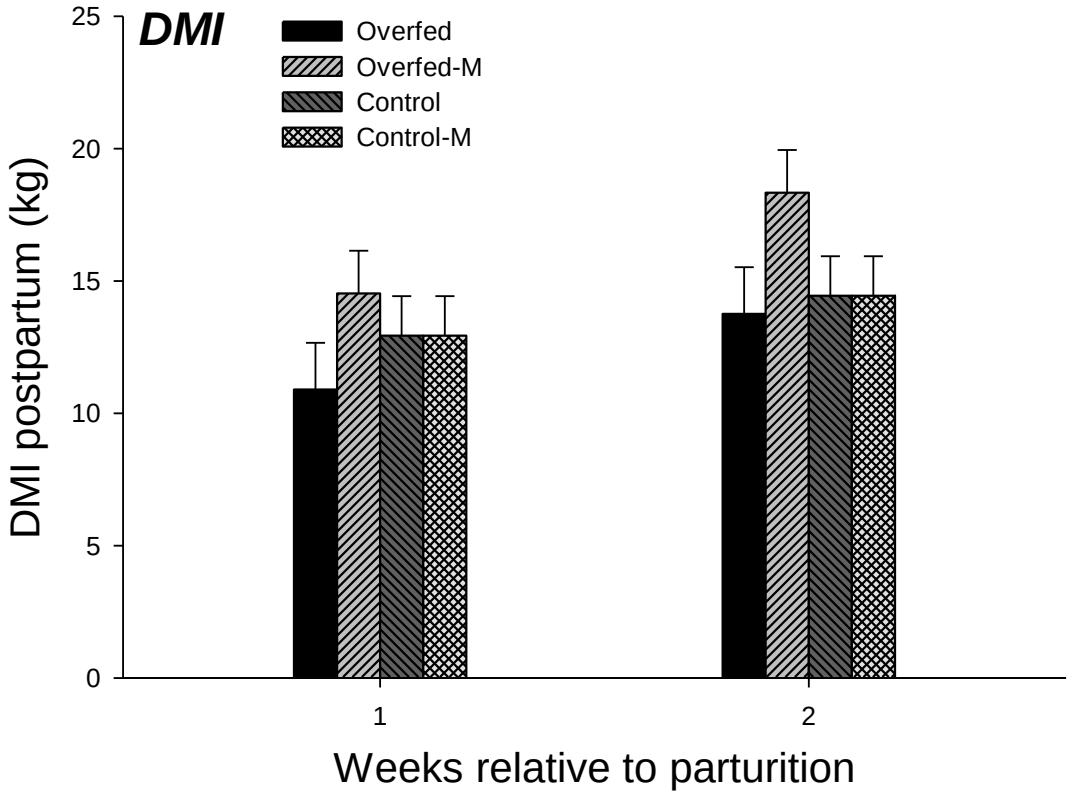

Figure 1.5. Mean weekly dry matter intake (DMI) in cows after parturition. Effects in model: time (P<0.01), diet (P=0.77), monensin (P=0.16), diet x monensin (P=0.26), diet x time (P=0.74), monensin x time (P<0.05).

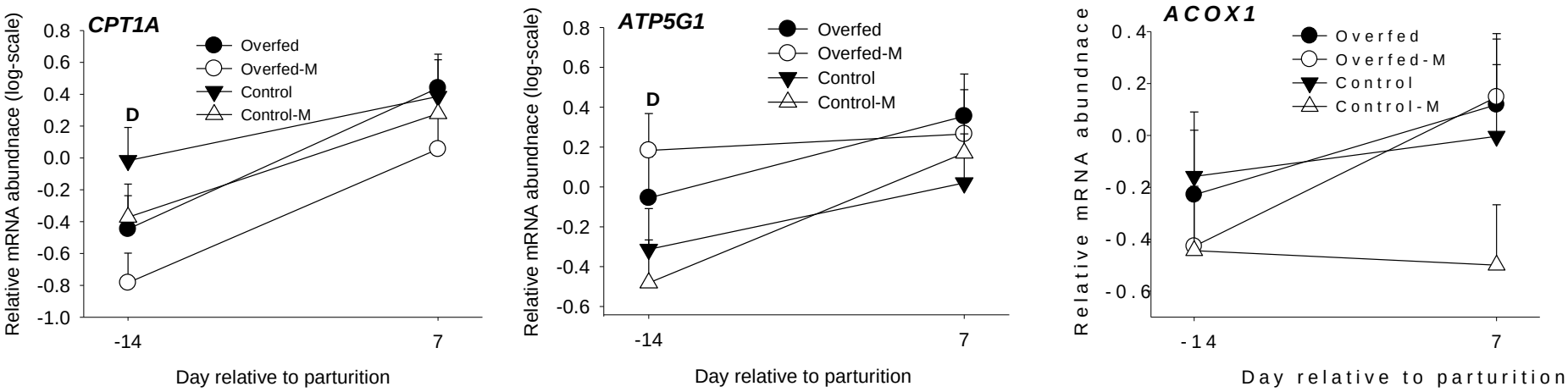

Figure 1.6. mRNA expression (log2-scale; mean ± SEM) of hepatic genes involved in fatty acid oxidation and ATP synthesis from cows in this experiment. D indicates an interaction effect of diet x time ($P<0.05$) at the specific time point. *CPT1A*: carnitine palmitoyltransferase 1A in liver; *ATP5G1*: ATP synthase, H+ transporting, mitochondrial Fo complex, subunit C1; *ACOX1*: acyl-CoA oxidase 1, palmitoyl

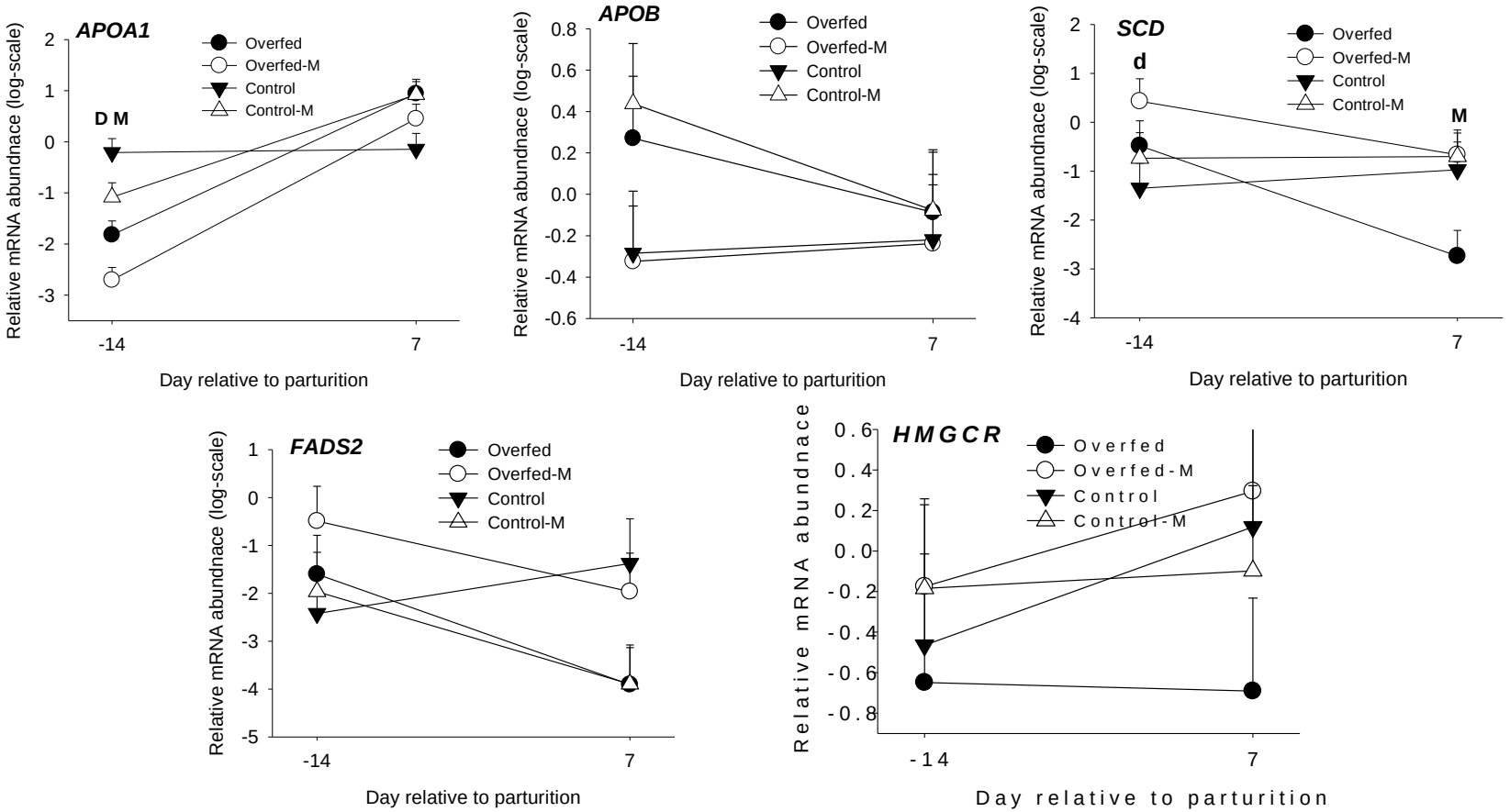

Figure 1.7. mRNA expression (log2-scale; mean ± SEM) of hepatic genes involved in lipid metabolism. D indicates an interaction effect of diet x time (P<0.05) at the specific time point. M indicates an interaction effect of monensin x time (P<0.05) at the specific time point; d indicates tendency of interaction of diet x time (P<0.10). *APOA1* : apolipoprotein A-I; *SCD*: stearoyl-CoA desaturase; *APOB*: apolipoprotein B; *FADS2*: fatty acid desaturase 2; *HMGCR*: 3-hydroxy-3-methylglutaryl-CoA reductase.

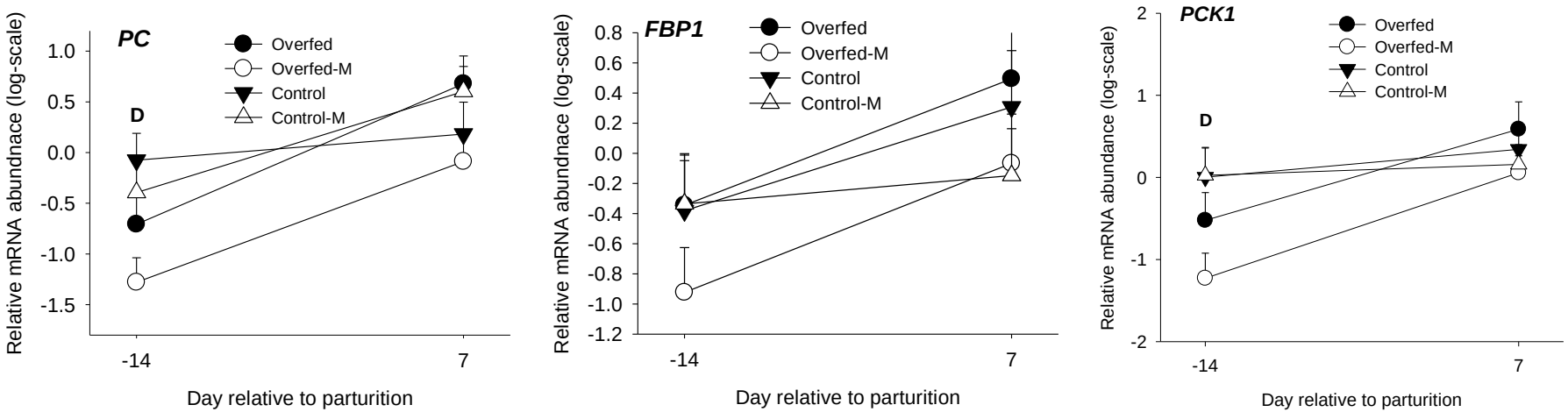

Figure 1.8. mRNA expression (log2-scale; mean ± SEM) of hepatic genes involved in gluconeogenesis from cows in this experiment. D indicates an interaction effect of diet x time ($P<0.05$) at the specific time point. *PC*: pyruvate carboxylase; *FBP1*: fructose-1,6-bisphosphatase 1; *PCK1*: Phosphoenolpyruvate carboxykinase 1.

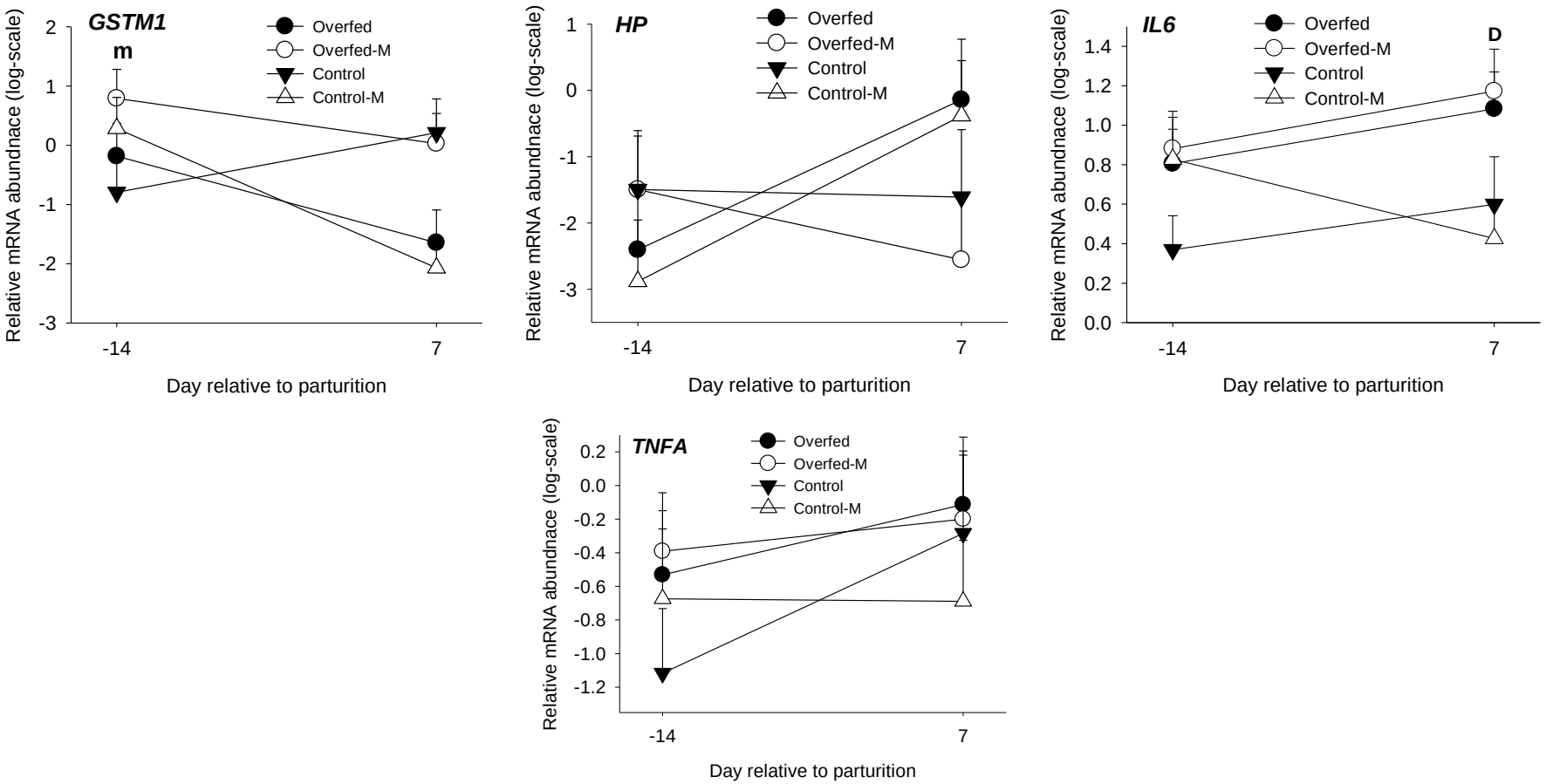

Figure 1.9. mRNA expression (log2-scale; mean ± SEM) of hepatic genes involved in inflammation, acute phase response, and detoxification/oxidative stress from cows in this experiment. D indicates an interaction effect of diet x time ($P<0.05$) at the specific time point. m indicates tendency of interaction of monensin x time ($P<0.10$). *IL6*: interleukin 6; *TNFA*: tumor necrosis factor alpha; *GSTM1*: glutathione S-transferase mu 1; *HP*: haptoglobin.

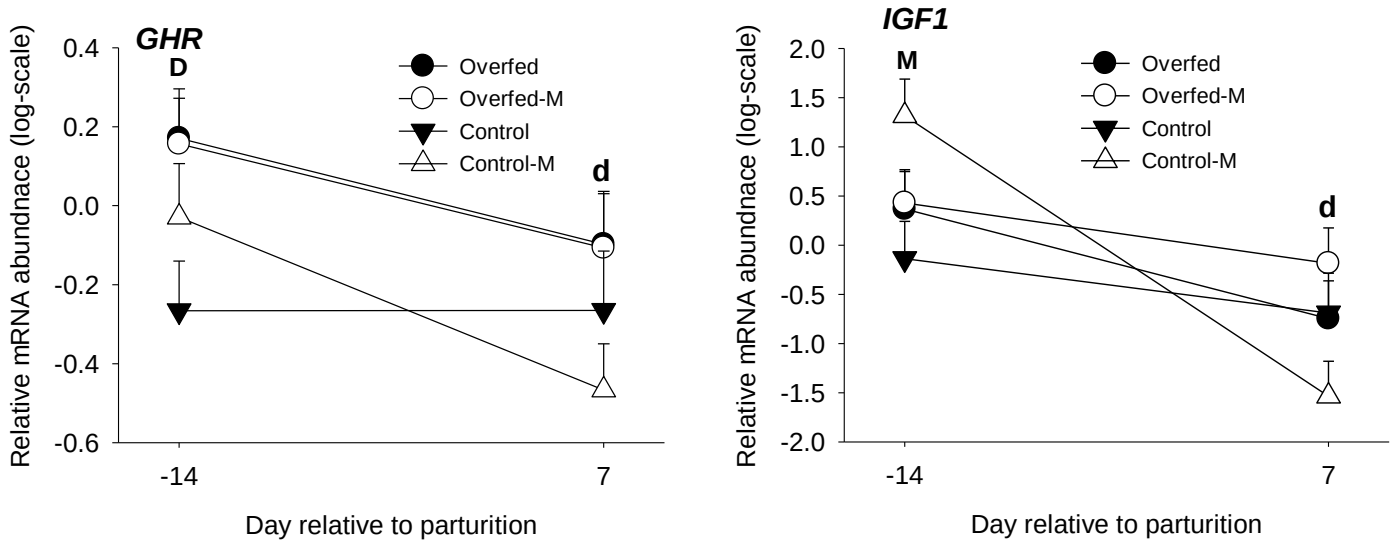

Figure 2.0. mRNA expression (log2-scale; mean ± SEM) of hepatic genes involved in growth hormone/IGF-1 axis from cows in this experiment. D indicates an interaction effect of diet x time ($P<0.05$) at the specific time point. M indicates an interaction effect of monensin x time ($P<0.05$) at the specific time point; d indicates tendency of interaction of diet x time ($P<0.10$). *GHR*: growth hormone receptor; *IGF1*: insulin-like growth factor I.

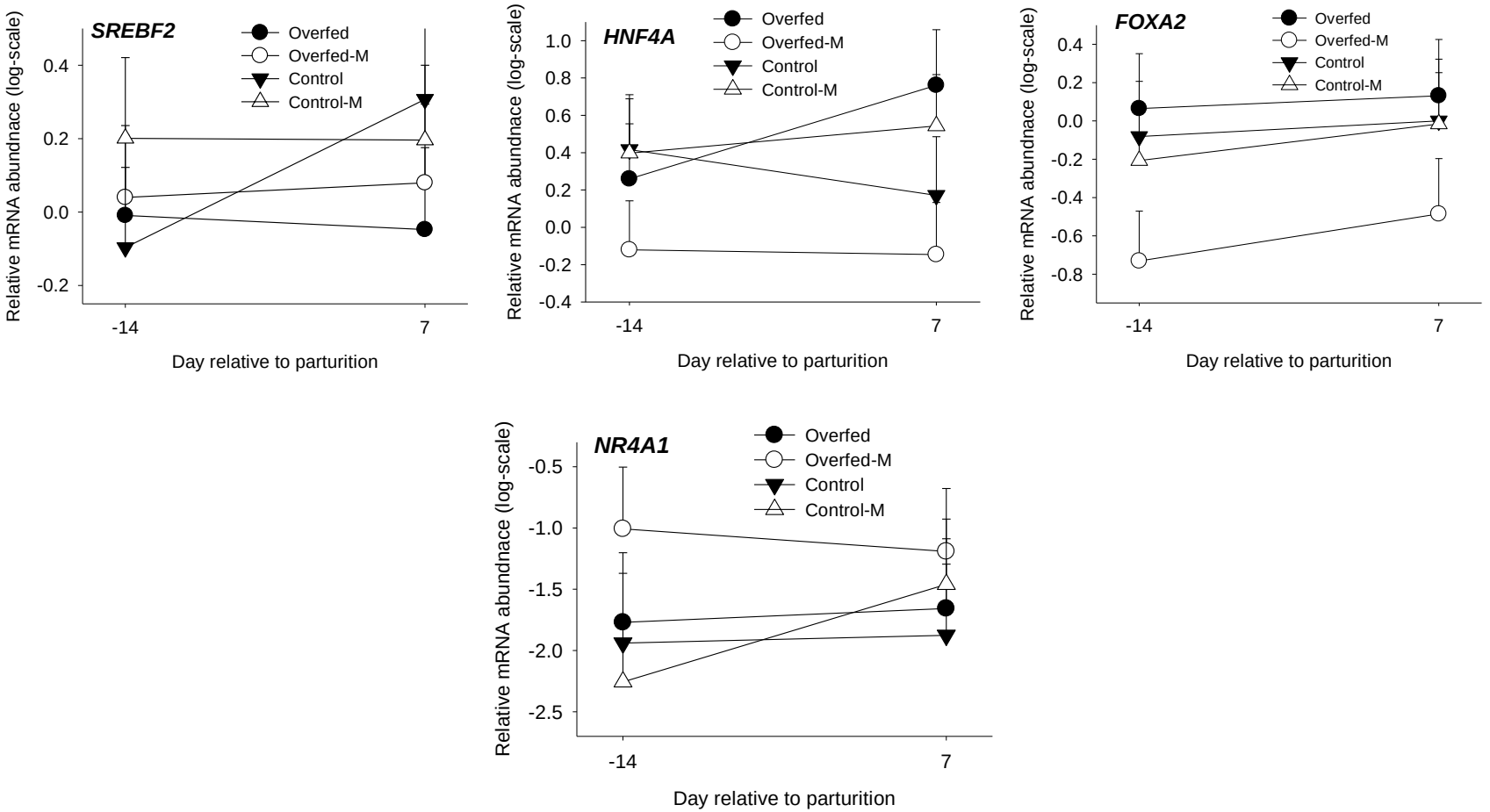

Figure 2.1. mRNA expression (log2-scale; mean ± SEM) of hepatic genes of transcription factors from cows in this experiment. *SREBF2*: sterol regulatory element binding transcription factor 2; *HNF4A*: hepatocyte nuclear factor 4, alpha; *FOXA2*: forkhead box A2; *NR4A1*: nuclear receptor subfamily 4, group A, member 1.

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